Stochastic simulation studies for honeybee breeding

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Abstract

This work shows the results of different simulation studies on honeybee breeding, aiming to find successful yet sustainable breeding strategies.

Chapter 1 gives a short introduction to animal breeding in general and how it can be described by mathematical models. In this context, the particular strengths of Monte Carlo simulation studies are explained. Furthermore, the chapter provides a review of basic honeybee biology and outlines the challenges in transferring general theory of quantitative genetics to this species.

There are standard computer programs which are used for stochastic simulations of animal breeding. But none of them is able to represent the biological properties of honeybees. Therefore, the program `BeeSim` was developed to fill this gap. *Chapter 2* describes the properties of the program.

When simulating honeybee populations, one has to make a choice if and how the genetics of individual bees should be simulated. Once one decides to model bees on an individual level, there are two main options for their genetics. The infinitesimal model assumes the genetics to be determined by an infinite number of loci, each with infinitesimally small influence on the traits of interest. It allows an individual's genetics to be simply described by only a few numbers. Alternatively, there are finite locus models, assuming a finite number of gene loci affecting the traits with possibly different magnitudes. *Chapter 3* shows the different behaviors of the two models in honeybee breeding simulations, leading to the conclusion that long-term simulation studies should rather rely on finite locus models in order to minimize the risk of underestimating the loss of genetic variety in the population.

Mating control is generally seen as a key factor of successful animal breeding. However, controlled mating is hard to achieve in honeybees, and many breeding organizations lack the necessary infrastructure. It is therefore tempting to breed by only selecting superior dam queens and neglecting the control of their mating behavior. The simulation studies of *Chapter 4* show that this approach cannot yield satisfactory genetic gain in the middle

to long-term and that mating control is crucial for reasons beyond avoiding unwanted introgression of other honeybee subspecies.

Chapter 5 shows the application of the `BeeSim` program to the question of optimal selection of honeybee populations in terms of sustainability. The sharper the selection procedure, the faster the genetic development of a breeding population in the desired direction. However, fast development comes with high inbreeding rates and a rapid loss of genetic variance. The optimal selection strategy depends on factors such as population size and heritabilities of the selection trait. The simulation studies of this chapter quantify these dependencies and thus evaluate competing selection approaches.

Chapter 6 provides a conclusion of the findings in the previous chapters as well as an outlook to what further promising investigations may be possible with the help of honeybee-specific Monte Carlo simulations in the future.

Chapters 3, *4*, and *5* have previously been published in peer-reviewed journals; the articles are referenced at the beginning of the respective chapters. In comparison with the published versions, the layouts were adjusted to achieve a uniform appearance. This includes formula notation and the display and numbering of tables and figures. The few cases where content was changed are clearly marked with explaining footnotes.

Zusammenfassung

Diese Arbeit vereinigt die Resultate mehrerer Simulationsstudien über die Honigbienenzucht mit dem Ziel, erfolgreiche und nachhaltige Zuchtstrategien zu finden.

In *Kapitel 1* wird eine kurze Einführung in die allgemeine Tierzucht gegeben. Zudem wird erklärt, wie diese mit mathematischen Modellen beschrieben werden kann. In diesem Kontext wird auch die Stärke von Monte-Carlo-Simulationen beleuchtet. Weiterhin liefert das Kapitel einen Überblick über wesentliche biologische Besonderheiten der Honigbiene und wie diese die Übertragung der Theorie quantitativer Genetik von anderen Nutztieren beeinflussen.

Keines der gängigen Computerprogramme, die für stochastische Simulationen von Zuchtpopulationen verwendet werden, kann die biologischen Besonderheiten der Honigbiene abbilden. Deswegen wurde das Programm `BeeSim` entwickelt um diese Lücke zu schließen. *Kapitel 2* beschreibt die Eigenschaften des Programms.

Bei der Simulation von Honigbienenpopulationen muss man sich für ein Modell entscheiden, das die Genetik der Bienen abbildet. Entscheidet man sich dafür, alle Bienen individuell zu simulieren, gibt es hierfür im Wesentlichen zwei Wahlmöglichkeiten. Das Infinitesimalmodell nimmt an, dass genetische Eigenschaften von einer unendlichen Anzahl Loci bestimmt werden, die alle einen infinitesimal kleinen Anteil daran tragen. Es erlaubt, die individuellen genetischen Eigenschaften mit wenigen Kenngrößen einfach zu beschreiben. Als Alternative gibt es Finite-Locus-Modelle. Diese gehen von einer endlichen Anzahl Loci mit Einfluss auf ein Merkmal aus, wobei die einzelnen Einflüsse unterschiedlich groß sein können. In *Kapitel 3* werden die unterschiedlichen Auswirkungen der Modelle in Simulationsstudien zur Honigbienenzucht dargestellt und geschlossen, dass Langzeitstudien eher auf Finite-Locus-Modellen basieren sollten um die Gefahr einer Unterschätzung des Verlusts genetischer Varianz zu minimieren.

Anpaarungskontrolle ist allgemein als ein Schlüssel zur erfolgreichen Tierzucht anerkannt. Allerdings ist sie bei Honigbienen nur schwer zu bewerkstelligen und vielen Zuchtverbänden fehlt die nötige Infrastruktur. Daher ist es naheliegend, für die Zucht lediglich die überlegenen Mutterköniginnen zu selektieren und ihr Paarungsverhalten zu ignorieren.

In *Kapitel 4* wird gezeigt, dass dieser Ansatz mittel- bis langfristig keine großen genetischen Zugewinne zu generieren vermag und dass Anpaarungskontrolle für eine erfolgreiche Zucht nicht nur zum Zwecke der Verhinderung ungewünschter Einkreuzungen wichtig ist.

Kapitel 5 zeigt die Anwendung des `BeeSim`-Programms auf die Frage nach optimalen Selektionsraten in der nachhaltigen Honigbienenzucht. Je schärfer selektiert wird, desto schneller verändert sich die Genetik der Population in die gewünschte Richtung. Allerdings, geht eine schnelle Entwicklung mit hohen Inzuchtraten und einem drastischen Verlust genetischer Varianz einher. Die optimale Selektionsstrategie hängt von vielen Faktoren, wie der Populationsgröße und Erblichkeiten der Selektionsmerkmale, ab. Durch stochastische Simulationen können diese Abhängigkeiten quantifiziert und die verschiedenen Zuchtansätze so evaluiert werden.

In *Kapitel 6* werden allgemeine Schlussfolgerungen aus den Ergebnissen der vorherigen Kapitel gezogen. Außerdem gibt das Kapitel einen Ausblick, welche weiteren Untersuchungen zur Honigbienenzucht mit Hilfe von Monte-Carlo-Simulationen in der Zukunft angestellt werden können.

Die *Kapitel 3*, *4* und *5* sind zuvor in begutachteten Fachzeitschriften veröffentlicht worden. Die entsprechenden Artikel werden jeweils am Kapitelanfang angegeben. Das Layout wurde im Vergleich zu den veröffentlichten Versionen angepasst um ein einheitliches Erscheinungsbild zu erreichen. Dies bezieht sich unter anderem auf die Notation in Formeln und die Darstellung und Nummerierung von Abbildungen und Tabellen. Die wenigen Stellen, an denen inhaltliche Änderungen vorgenommen wurden, sind klar mit erklärenden Fußnoten markiert.

Selbstständigkeitserklärung

Hiermit erkläre ich, die Dissertation selbstständig und nur unter Verwendung der angegebenen Hilfen und Hilfsmittel angefertigt zu haben. Ich habe mich anderwärts nicht um einen Doktorgrad beworben und besitze keinen entsprechenden Doktorgrad. Ich erkläre, dass ich die Dissertation oder Teile davon nicht bereits bei einer anderen wissenschaftlichen Einrichtung eingereicht habe und dass sie dort weder angenommen noch abgelehnt wurde. Ich erkläre die Kenntnisnahme der dem Verfahren zugrunde liegenden Promotionsordnung der Lebenswissenschaftlichen Fakultät der Humboldt-Universität zu Berlin. Weiterhin erkläre ich, dass keine Zusammenarbeit mit gewerblichen Promotionsbearbeiterinnen/Promotionsberatern stattgefunden hat und dass die Grundsätze der Humboldt-Universität zu Berlin zur Sicherung guter wissenschaftlicher Praxis eingehalten wurden.

Statement of authorship

I hereby declare that I completed the doctoral thesis independently based on the stated resources and aids. I have not applied for a doctoral degree elsewhere and do not have a corresponding doctoral degree. I have not submitted the doctoral thesis, or parts of it, to another academic institution and the thesis has not been accepted or rejected. I declare that I have acknowledged the doctoral degree regulations which underlie the procedure of the Faculty of Life Sciences of Humboldt-Universität zu Berlin. Furthermore, I declare that no collaboration with commercial doctoral degree supervisors took place and that the principles of Humboldt-Universität zu Berlin for ensuring good academic practice were abided by.

Table of contents

Chapter 1

Animal breeding and honeybees

1.1 Livestock breeding

The general aim of selective breeding endeavors is the gradual genetic improvement of a population over the course of several generations [1]. Here, genetic improvement is to be seen with respect to one or several quantitative traits such as milk yield in cattle, egg-laying frequency in chicken, or honey yield in honeybees. The improvement is achieved by a selection regime of breeders which ideally allows only individuals with desirable genetic features to reproduce. Some of the major concerns in breeding activities can be summarized under the catchwords *detection*, *decision*, *control* and *sustainability*:

- *Detection.* In order to select genetically superior individuals, one first needs to identify them. The most important factors to detect the genetic quality of an animal are its phenotypic features. Therefore, animal breeding inevitably requires record-keeping of the individuals' phenotypes. While for some traits, like slaughter weight in pigs, the attainment of these records is straightforward, other traits, like gentleness in honeybees, require the development of a standardized measuring methodology in order to obtain comparable results from different breeders [2]. However, an individual's phenotype is determined not only by its genes but also by its environment and further random effects. In the last 100 years, mathematical theory galore has been developed to isolate the genetic contribution to the phenotype and thus enable breeders to select those individuals which are of true genetic quality and not just those which potentially benefited from favorable environmental conditions [3].

- *Decision.* In most cases, breeders do not follow a singular breeding goal but rather a collection of several objectives. When an individual excels in some traits while having deficits in others, trade-off decisions have to be made in order to determine whether that individual is fit for reproduction [4, 5]. In dairy cattle, for example, one is interested in fertile cows, because having offspring is a prerequisite for milk production. Furthermore, one is also interested in high milk yields per lactation. Unfortunately, these two traits have been shown to be negatively correlated—selection for one trait can thus easily result in negative selection for the other trait [6]. Therefore, a well-calibrated balance between selection foci needs to be found. Similar considerations apply when a trait is not determined by an individual alone but also by contemporaries. The weaning weight of piglets is affected by their own ability to put on weight as well as their mother's ability to feed them. Such direct and maternal genetic effects are in many cases negatively correlated; sows which are exceptionally good at feeding their offspring often have piglets that struggle in gaining weight by themselves [7].

- *Control.* Once genetically superior individuals are detected, it must be secured that only these individuals are allowed to reproduce. Consequently, mating in the population must be controlled. Practices of mating control, such as the castration of large numbers of males, are probably as old as agriculture itself [8]. In many agricultural species, practices have been developed further over the years, culminating in modern strategies involving artificial insemination with sexed sperm and embryo transfers [9]. In some species, like aquaculture and honeybees, controlled mating is especially hard to achieve because the mating process cannot easily be observed or steered.

- *Sustainability.* Intense selection will only allow relatively few individuals to reproduce. The selected individuals thus each have to produce large numbers of offspring in order to maintain a constant population size. Therefore, many individuals in the breeding population will be closely related, thereby narrowing down the genetic variance within the population. This variance, however, is a main prerequisite for further genetic improvement [10]. Moreover, it has been shown that increasing rates of inbreeding have negative fitness effects on individuals, the so-called inbreeding depression [11]. Therefore, a successful breeding scheme has to take questions of sustainability into account. It has to generate genetic improvement and at the same

time maintain genetic diversity in order to allow for further improvements in the future [12].

This list of breeding considerations is by no means exhaustive. Cost-efficiency and animal welfare are examples of further concerns of animal breeders that are not covered. But the presented four aspects—*detection*, *decision*, *control*, and *sustainability*—are of undeniable importance and mark the areas to which this work aims to contribute in the field of honeybee breeding.

1.2 Mathematical models

Tackling the aforementioned four challenges of animal breeding is much facilitated by mathematical models. Two of the most important models in animal breeding are the so-called *animal model* and the model of *Mendelian inheritance*. They provide access to two integral questions of breeding-related biology:

- How are phenotype and genotype of an individual related?
- How are genotypes passed from parents to their offspring?

The first of these questions touches the breeding aspects of *detection* and *decision*, while the second question affects the topics of *control* and *sustainability*. These connections are discussed in the following two sections.

1.2.1 The animal model

The animal model gives a description how individual phenotypes are determined by a number of components. In its simplest form, it is described by the formula [13]

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}\mathbf{u} + \mathbf{e}. \tag{1.1}$$

It assumes that the vector of phenotypic records $\mathbf{y}$ depends linearly on effects of the different environments $\mathbf{b}$, the individual genetics $\mathbf{u}$, and residual effects $\mathbf{e}$. The matrices $\mathbf{X}$ and $\mathbf{Z}$ match the respective environments and genetics to the phenotypes. The following example illustrates the model.

Example 1. Assume four cows, c_1 to c_4, living in two different environments, A and B, and producing different amounts of milk over the course of one lactation (6000, 6500, 7000,

and 8000 kg, respectively). The animal model provides a decomposition of the vector $\mathbf{y}$ of lactation yields which may for example look as follows:

$$\begin{bmatrix} 6000 \\ 6500 \\ 7000 \\ 8000 \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ 1 & 0 \\ 0 & 1 \\ 0 & 1 \end{bmatrix} \begin{bmatrix} 5700 \\ 8000 \end{bmatrix} + \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} 200 \\ 800 \\ -700 \\ 100 \end{bmatrix} + \begin{bmatrix} 100 \\ 0 \\ -300 \\ -100 \end{bmatrix}. \tag{1.2}$$

I. e., while environment A (with cows c_1 and c_2) normally allows for a milk yield of 5700 kg, the more favorable environment B on average allows for 8000 kg. Cows with the genetic properties of cow c_2 can be expected to produce 800 kg more milk than the average, while cows with the genetics of cow c_3 can be expected to yield 700 kg less than the average. The vector $\mathbf{e}$ finally covers, how far the individual realization differs from these expectations due to further influences that were not explicitly covered. In particular it is worth noting that in the example, cow c_4 with the best phenotype is different from the cow with the most favorable genetics, which is c_2.

Naturally, when given a vector $\mathbf{y}$ of phenotypic records, the question arises, how a concrete realization of the decomposition in Equation 1.1 looks like. An answer was provided by C. R. Henderson who developed the methodology of best linear unbiased prediction (BLUP) [14]. This procedure yields likely values for the vectors $\mathbf{b}$ and $\mathbf{u}$ using a linear regression based on *a priori* assumptions on the genetic covariances. In particular, it assumes that genetic similarity translates directly to correlations between the genetic values u_i and u_j of two individuals i and j. Traditionally, rates of genetic similarity are deduced from the pedigree [15]; however, there are modern approaches to detect the similarity more directly by explicitly reading genetic marker information from the DNA [16]. The animal model and the BLUP methodology of genetic evaluation are closely connected and often mentioned in the same breath [17–19]. The mathematical formulation of splitting phenotypic values into an environmental part and a genetic part as in Equation 1.1 has proven highly valuable in the *detection* of superior individuals in a breeding population.

Like any mathematical model, Equation 1.1 is a simplification of reality. For example, it does not cover non-linear contributions or interactions between environmental and genetic effects. However, in many cases the model can easily be extended to allow for generalizations such as the presence of maternal effects. Recalling the example of the weaning weight in pigs, a specific genetic set-up can have two different genetic values depending

on whether it is expressed in the growing piglet or in the feeding sow. The animal model can thus be extended to the form

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_{\text{mat}}\mathbf{u}_{\text{mat}} + \mathbf{Z}_{\text{dir}}\mathbf{u}_{\text{dir}} + \mathbf{e}, \tag{1.3}$$

where $\mathbf{u}_{\text{mat}}$ and $\mathbf{u}_{\text{dir}}$ represent the maternal and direct genetic values [13]. In Equation 1.2 the matrix $\mathbf{Z}$ was the identity matrix, linking individuals' genotypes to their phenotypes. While this remains the case for $\mathbf{Z}_{\text{dir}}$, the matrix $\mathbf{Z}_{\text{mat}}$ connects the genotypes of dams to the phenotypes of their offspring. Applying the BLUP methodology to the extended model of Equation 1.3 provides each individual with estimated breeding values for the direct and the maternal effects. The absolute breeding value may then be defined as the sum of direct and maternal breeding values, thus comprising the complete potential for genetic contributions to further generations [20]. In case of multiple selection traits, similar extensions of the animal model can be applied [21]. Thus extended, the animal model facilitates *decisions*, allowing for a judgement of different genetic qualities of single individuals.

1.2.2 Mendelian inheritance

The model of additive Mendelian inheritance describes how genetic properties, i. e. breeding values, are passed from parents to offspring. In its simplest form it can be written as [10]

$$u_{\text{offspring}} = \frac{1}{2}u_{\text{dam}} + \frac{1}{2}u_{\text{sire}} + \delta. \tag{1.4}$$

This signifies that on average, an offspring can be expected to inherit the mean genetic values of its parents. However, due to the fact that each parent only passes half of its genetic information, there is some sampling variation which is represented by the term δ. Again, the general Equation 1.4 is illustrated by an example:

Example 2. In dairy cattle, we consider a bull b and a cow c which are sire and dam of two offspring o_1 and o_2. The respective breeding values of b and c for yearly milk yield are 7000 kg and 8000 kg. (Note that despite not producing milk themselves, the genetic properties of a bull can still be associated with a value for this trait.) Then, the genetic

values of the offspring may be

$$u_{o_1} = \frac{8000}{2} + \frac{7000}{2} + 750 = 8250,$$
$$u_{o_2} = \frac{8000}{2} + \frac{7000}{2} - 550 = 6950. \tag{1.5}$$

In particular, children of the same parents do generally not carry the same genetic properties: The variance of the Mendelian sampling term δ signifies how much the genetics of full-siblings can vary.

Equation 1.4 serves as the starting point for many derivations in quantitative genetics. It can be used to connect degrees of relationship with genetic similarity [15] or to predict average genetic properties of a new generation from the breeding values of the selected dams and sires [22]. However, Mendelian inheritance as it is modelled in Equation 1.4 can only be used to describe an offspring's genotype if both parents are known. This further strengthens the importance of *control* for animal breeding: Not only is it paramount that only genetically desirable individuals reproduce, but in order to give a sound mathematical description of inheritance, it is also important to control (or at least record) which individual matings take place.
In Fisher's infinitesimal model of genetics [23], the variance of the Mendelian sampling term δ can be expressed in terms of the genetic variance of the trait and the parents' inbreeding coefficients [24]. By this nature, the development of δ over time provides an implicit description of the *sustainability* of a breeding program. Genetic progress over generations is only possible if there are offspring which have better genetic properties than their parents and the extent to which this will be the case is determined by the variance of δ. So if the variance of δ declines over time due to inbreeding or genetic drift, this indicates limited sustainability of a breeding program.

1.2.3 Monte Carlo simulations

Separate explanations of the animal model and the model of Mendelian inheritance, as they were given in the two previous sections, must remain vague because the models develop their true strengths only when used in combination:
Making use of the animal model, genetically superior individuals are detected and selected for reproduction. When reproducing, they pass their genetic values to their offspring according to the Mendelian rules. For the next round of selection, the genetic similarity

of individuals can be determined by investigating which ancestors they have in common and which part of their genetics—inherited according to Equation 1.4—they thus share. This information in turn serves as an input for the BLUP methodology. Hence, an adequate description of breeding over multiple generations requires iterative usage of the animal model and Mendelian inheritance. Explicit mathematical descriptions of breeding processes, such as Lush's *breeder's equation*, are often limited to few generations [22], because processes over many generations are complicated by multiple interactions between the involved models. Most analytical descriptions of multi-generational breeding could only be obtained under several simplifying assumptions [25–27].

However, the vast increase in capacity of computers during the last decades allowed for an alternative to analytic descriptions of breeding processes, namely Monte Carlo simulation studies. Presently, modern computer programs, such as ADAM [28], can simulate thousands of individuals with their respective genetic set-ups. The simulated animals' genetics are inherited according to the Mendelian rules, while phenotypes and estimated breeding values are generated by use of the animal model. Through the explicit use of computer-generated random values, e. g. for the Mendelian sampling terms δ, complicated dependencies, like those between individual relationship coefficients and breeding values, no longer have to be deduced theoretically. Rather, they can directly be inferred from the outcomes of the simulations.

Nowadays, Monte Carlo simulation studies are ubiquitous in animal breeding. They are used in a wide range of contexts: to validate the benefits of newly designed breeding schemes [29], predict the extent of evaluation biases [30], or investigate the influence of incomplete information [31].

1.3 Adaptation to honeybees

1.3.1 Honeybee breeding

So far, we outlined a theoretical framework of livestock breeding in general. In the following we will turn to the special case of the honeybee *Apis mellifera*. Currently, honeybees are kept and bred all over the world. Traditionally, there were four main traits of interest in honeybee breeding:

- *Honey yield.* For most beekeepers, honey production is the main incentive for their work. Consequently, there is a keen interest in honeybee colonies which produce

large amounts of honey. The trait is relatively easy to access by weighing combs before and after honey extraction [2]. Depending on the subspecies, a yearly honey yield between 10 kg and 40 kg can be considered as normal. In particular through migratory beekeeping, much higher yields of up to 200 kg can be reached [32]. In theory, the honey yield of a colony could also be negative if the beekeeper has to feed more honey than s/he can harvest in a season. However, such situations are rarely reported.

- *Gentleness.* Gentleness of honeybees describes a reduced defensive behavior towards humans. This trait not only enhances the manageability of the hive by the beekeeper but also the acceptance of beekeeping in populated areas. According to the most prevalent testing protocol, it is rated by the breeder several times a season on a scale from 1 to 4, ranging from "bees show no sign of aggression" to "bees attack despite use of smoke" [2, 33]. Despite efforts to establish well-defined categories for the individual ratings, this trait always depends on the subjective judgment of the breeder.

- *Calmness.* Successful beekeeping requires repeated inspections of the hive. These inspections are much facilitated if the bees remain calmly on their combs rather than fly around. Therefore, calmness on the comb during inspections has been made out as a breeding goal. Like gentleness, calmness is usually rated on a scale from 1 to 4 [2].

- *Swarming behavior.* When a honeybee queen becomes old or a colony perceives itself as too big, a new queen can be produced and the old queen will leave the hive together with a large group of worker bees, the so-called *swarm*. This behavior is unwanted by most beekeepers because swarms have to be caught again and honey production is negatively affected [34]. Therefore, honeybees are actively selected against the tendency for swarming. Like the two aforementioned manageability traits, swarming tendency is typically rated on a scale from 1 to 4 [2].

In recent years, defense against the parasitic mite *Varroa destructor* has become increasingly important and thus recognized as a desirable breeding trait [35, 36]. *Varroa*-defense can be measured in various different forms, including the workers' ability to remove infected brood and the growth of the mite population over the season [2]. Most European breeding programs select for an index trait made up from these aspects [37]. Regionally,

further breeding goals may be pursued, for example royal jelly production in France [38] and China [39] or resistance against the Sacbrood virus in South Korea [40].
Honeybee breeding differs from that of other species in several aspects. The most striking of these differences is that in honeybees, being social insects, traits are generally not expressed by individuals but rather in a collective, the colony. As will be described later on, the honeybee's biological peculiarities complicate the mathematical description of breeding processes. Hence, in comparison to other livestock species, it appears that honeybee breeding is lagging behind between one and two decades. While Henderson's BLUP methodology was applied in cattle from the early 1980s onward [41], its adaptation to honeybees was not implemented until the mid-1990s [37] and while genomic marker information has regularly been used in cattle breeding since ca. 2005 [42], the necessary SNP-chip for honeybees has only recently been developed [43].
Historically, active breeding of honeybees has been limited to few European subspecies like *A. m. carnica* and *A. m. ligustica*. Consequently, these subspecies became increasingly attractive to beekeepers all over the world. Other honeybee species and subspecies are therefore increasingly endangered by replacement or hybridization [44, 45]. Recently, in the course of the European project *SmartBees*, several breeding programs for endangered populations are burgeoning. In order to be successful, they require meticulous planning and adequate theoretical background considerations, tailored to the specific situations.

1.3.2 Honeybee biology

A basic understanding of honeybee biology is essential for describing breeding processes of this species mathematically. These necessities will be provided in this section.
Honeybees live in colonies of several thousand individuals, each of which belongs to one of three different castes [46]:

- *Queens.* The queen is the only female honeybee in the hive which is able to reproduce. All other members of the colony are her offspring. The development of a female larvae into a queen is induced by a special diet, consisting in large parts of royal jelly. Shortly after hatching, a queen will perform one or several nuptial flights during which she mates in the air with several drones (male honeybees) from hives in the vicinity. The drones' semen is henceforth stored in the queen's spermatheca and used to fertilize eggs for the rest of the queen's life. Queens can regulate work-

ing processes in the hive by emitting pheromones and thereby contribute to most quantitative traits. They are diploid organisms and can live for several years.

- *Worker bees.* With up to 60.000 individuals per hive, worker bees constitute the majority of a colony. They are female and diploid but generally do not reproduce. They perform most of the tasks in the hive, whence they directly contribute to all traits.

- *Drones.* Male honeybees are called drones. Unlike queens and worker bees, they are haploid and develop from unfertilized eggs. Since they do not contribute to the chores in the hive but only play a role in reproduction, they are often called *flying gametes*.

The honeybee queen's polyandry, i. e. her mating with multiple drones, yields serious problems for breeders as far as *control* is concerned. While artificial insemination of a queen with a single drone is technically possible, the resulting colonies do not develop to full strength and most of the time cannot be overwintered successfully [47]. Thus, full control over which specific drone is a queen's sire can in general not be given. Partial control, however, is indeed possible and can be achieved by making sure that the area of the queen's nuptial flight is void of drones with the exception of drones from few selected colonies. This is typically the situation on *isolated mating stations* which are placed in mountain valleys or on islands.

1.3.3 Honeybee models

When applying genetic models to the honeybee, several adjustments are necessary to cover the biological properties correctly. This becomes obvious when looking at two female offspring of the same queen. In mammals, two offspring of the same dam have a quarter or half of their genes in common, depending on whether they share the same sire or not. If two offspring from a honeybee queen have different sire drones, they will also share a quarter of their genetics. However, since drones are haploid, all their sperm cells are genetically identical. Therefore, honeybees that stem from the same drone sire are much closer related than it is the case in mammals: they share 75% of their genes.
As described earlier, honeybee breeders are interested in entire colonies rather than individual worker bees. Based on works of Chevalet and Cornuet [48], Bienefeld and Pirchner [49] developed a model that comprises three entities: queens, worker groups, and pseudo

sires, the last-mentioned comprising the collective of drone producing queens on a mating station. The model of Mendelian inheritance (Equation 1.4) was then adjusted to account for the honeybee's genetic properties. This model was later improved by Brascamp and Bijma [50] to fully account for the collective nature of worker groups, haploidy of drones, and different probabilities of individual sirehoods.
This classification of honeybees into queens, worker groups and pseudo sires allowed for an adaptation of the BLUP animal model to the honeybee [19]. In the situation of Equation 1.3, the phenotype of a colony is influenced by maternal effects of the queen and direct effects of the worker group. The breeding value estimation for honeybees which is thus made possible has proven to be highly successful in the last two decades [37].

1.4 References

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Chapter 2

The program `BeeSim`

The program described in this chapter has been published on Dryad:[1]

M. Plate, R. Bernstein, A. Hoppe, K. Bienefeld Data from: Comparison of infinitesimal and finite locus models for long-term breeding simulations with direct and maternal effects at the example of honeybees. Dryad. Dataset, 2019.
`https://doi.org/10.5061/dryad.1nh544n`

It can be downloaded directly via
`https://datadryad.org/stash/downloads/file_stream/43054`.

[1]The program was published under the first author's birth name *Plate*.

2.1 Program description

The computer program `BeeSim` has been written to provide stochastic simulations of honeybee breeding processes. It is loosely inspired by the program ADAM [1] for other livestock species but was written from the ground up using the programming language C. The program simulates breeding programs which can be specified by the user via parameter files. Different breeding strategies can then be evaluated by comparing the respective outcomes in terms of e. g. inbreeding rates or genetic gain. The program simulates queens, drones and worker groups individually as they inherit genetic properties through multiple generations. Hereby, it respects honeybee specific features, such as haplo-diploid genetics and polyandry. It enables selection and keeps track of mutual relationship coefficients.
The user can make choices regarding the following parameters:

- *Genetic model.* The user can chose between a *finite locus model* and the *infinitesimal model* for the bees' genetics. While the finite locus model represents individual genes or markers, the infinitesimal model reflects a large number of loci, all contributing equally to a trait. In either model, the user can specify a trait by giving its genetic and residual variances. Queen and worker effects can be represented. In case of the finite locus model, different distributions for allele frequencies and allele effects can be specified as well as different linkage rates between loci. The program can furthermore be used to simulate historical populations in order to create linkage disequilibrium. At the moment, the program is limited to additive genetic inheritance.

- *Population.* The user may specify the population size in terms of colonies per year. In addition to the breeding population, a passive population may be simulated which is not actively selected but potentially interdepends with the breeding population via the exchange of queens and/or drones. Rates, to which extent such genetic exchange may take place, are also specified by the user. The population parameters also include minimum and maximum ages of queens to produce drones or queens, as well as potential culling ages. All population parameters can either be specified for the whole simulated time or individually for each simulated year.

- *Mating.* The user can specify how queens of the breeding and passive population should mate. Generally, there are three possibilities: (i) free mating, (ii) mating with drones from selected colonies via artificial insemination, or (iii) mating on

isolated mating stations. It is also possible, to let different parts of the population follow different mating strategies. For either mating, the user can decide how many drones should be involved in the process. For the isolated mating stations, several characteristics can be specified. These include the number of drone producing queens on the mating station and whether or not they are related, as well as the mating station security, i. e. how probable it is that a drone involved in a mating does actually come from one of the designated drone producing hives.

- *Selection.* The user can specify at which rates queens should be selected to produce new queens, mating stations, or drones for artificial insemination. Furthermore, it can be specified, how many queens from the same sister group can maximally be selected, thus enabling within- and across-family selection. Selection can follow four different criteria:
  - *phenotypical selection*, i. e. the queens whose colonies show the best phenotype are selected.
  - *genotypical selection*, i. e. the queens which can pass on the best genes are selected
  - *random selection*
  - *selection after genetic evaluation.* The selection scheme can employ a breeding value estimation. The program is designed to run with the BLUPF90 family of programs [2] for which the inverse relationship matrix is calculated by the program `PInCo` [3]. But alternative programs may easily be included, for example to feature genomic breeding strategies.

The program is continuously updated and expanded to feature further aspects of honeybee breeding.

2.2 Motivation of performed simulation studies

As follows from the program description, a large number of parameters can independently be chosen, thus giving rise to a plethora of possible simulation studies. In the remainder of this chapter, I will motivate why I decided to perform the specific investigations described in the following chapters.

The ultimate goal of my endeavors was to use the `BeeSim` program to find suitable breeding strategies for honeybee populations that provide sustainable genetic gain. For this purpose, it was crucial to first develop an understanding of the behavior of the used models in order to make reasonable choices. The first study, which is described in Chapter 3, therefore gave a broad survey how long-term developments depend on the chosen models and parameters. From the results, best practices for further more specialized Monte Carlo simulations could be deduced. In particular, the results indicated which genetic models are the most adequate for long-term breeding simulations.
After a suitable genetic model for the intended long-term simulations was found, in the next step I needed to define an adequate selection strategy. The biggest difference between selection schemes that are currently in practical use in Europe is the handling of mating control. While some breeding organizations invest great effort into the provision of secure mating stations [4], others let their queens mate freely [5]. I therefore conducted a study in which the consequences of these two strategies are investigated in detail. The results—which speak highly in favor of controlled mating—are presented in Chapter 4.
Based on the results of Chapters 3 and 4, the qualitative choices of genetic model and breeding system were determined for the final study. Chapter 5 provides applicable quantitative results regarding optimal selection rates. The outcomes of this study thus give concrete instructions to breeders and breeding organizations regarding an optimal design of their selection strategies.

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Chapter 3

Comparison of infinitesimal and finite locus models

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together with three appendices:

S1 Appendix: QTL effect analysis
`https://doi.org/10.1371/journal.pone.0213270.s001`

S2 Appendix: Linkage simulations
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S3 Appendix: Selection strength on direct and maternal effects
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[1]The article was published under the first author's birth name *Plate*.

Abstract

Stochastic simulation studies of animal breeding have mostly relied on either the infinitesimal genetic model or finite polygenic models. In this study, we investigated the long-term effects of the chosen model on honeybee breeding schemes. We implemented the infinitesimal model, as well as finite locus models, with 200 and 400 gene loci and simulated populations of 300 and 1000 colonies per year over the course of 100 years. The selection was of a directly and maternally influenced trait with maternal heritability of $h_m^2 = 0.42$, direct heritability of $h_d^2 = 0.27$, and a negative correlation between the effects of $r_{md} = -0.18$. Another set of simulations was run with parameters $h_m^2 = 0.53$, $h_d^2 = 0.34$, and $r_{md} = -0.53$. All models showed similar behavior for the first 20 years. Throughout the study, we observed a higher genetic gain in the direct than in the maternal effects and a smaller gain with a stronger negative covariance. In the long-term, however, only the infinitesimal model predicted sustainable linear genetic progress, while the finite locus models showed sublinear behavior and, after 100 years, only reached between 58% and 62% of the mean breeding values in the infinitesimal model. While the infinitesimal model suggested a reduction of genetic variance by 33% to 49% after 100 years, the finite locus models saw a more drastic loss of 76% to 92%. When designing sustainable breeding strategies, one should, therefore, not blindly trust the infinitesimal model as the predictions may be overly optimistic. Instead, the more conservative choice of the finite locus model should be favored.

3.1 Introduction

A major concern in sustainable breeding and conservation programs is the preservation of genetic variance in the population [1–4]. To estimate the development of genetic variance under various conditions, Monte Carlo simulations have been widely applied in animal breeding and conservation genetics since computers were introduced [5, 6], and they remain a valuable tool [7, 8]. Currently, two main types of genetic models are used to investigate developments in genetic variance via simulations: Fisher's infinitesimal model [9, 10] and the finite locus models [11, 12]. The infinitesimal model assumes that quantitative traits are genetically influenced by an infinitely large number of loci, each of which has the same infinitesimally small impact. In contrast, finite locus models assume a possibly large but finite number of loci contribute to a trait and allow for different magnitudes of influence of the respective loci. Both the infinitesimal model [13, 14] and finite locus models [15, 16] have been used in recent simulation studies.

Although existing software, such as ADAM [17], can run simulations based on either model, comparisons of the two models' properties in stochastic simulations appears to be scarce in the literature. Fournet-Hanocq and Elsen [18] compared Monte Carlo simulations based on the finite locus model with two deterministic simulations, one of which relied on the infinitesimal model, and found greater losses of genetic variance in the finite locus models. Further studies focused on only one of the models and found dependencies of the simulation outcomes on the population size [19] or the distribution of QTL effects [12]. In [20], simulations using both models were performed. However, this study compared the accuracy of different methods for breeding value estimation depending on the models rather than the properties of the models themselves. We are not aware of any direct comparison of Monte Carlo simulations based on the respective models.

Estimates of the long-term effects of animal breeding, especially the limits of selection possibilities, were introduced in [21], relating the possible total genetic gain to the effective population size. The authors of [19] studied the long-term behavior of Monte Carlo simulations that relied on the infinitesimal model. They found the problem of decreasing genetic variance in very small populations and expressed interest in similar studies with different genetic models. A study of long-term breeding effects involving a finite locus model was conducted in [22]. Here, the authors discovered the possibility of improving long-term responses by enhancing the selection of favorable minor alleles. Breeding schemes can select for either single traits or several intercorrelated traits. Often, these traits are not

only determined by single individuals alone but also by their contemporaries via indirect effects [23, 24]. The most prevalent of these indirect effects are probably the so-called maternal effects in which the genetic properties of a dam influence the performance of her offspring [25–27].

Simulation studies have already examined the effects of selective breeding for multiple traits and found that breeding value estimation should consider the correlation between different traits [28, 29]. Traditionally, most of these investigations have been solely based on the infinitesimal model [28, 30, 31]. Most modern finite model-based single trait simulation studies assume that there is a favorable allele and suppose that the allele's effects follow a heavy-tailed distribution, often realized by a gamma distribution. These assumptions cannot be easily transferred to a multivariate setting. Nevertheless, a number of multi-trait studies with finite locus models exist [29, 32, 33]. However, no common standard methodology has been established so far [34].

Simulation studies that include indirect effects appear to be almost exclusively based on the infinitesimal model [35–39]. It has been found that indirect effects have to be correctly addressed in the breeding value estimation to obtain optimal results and that a negative correlation between direct and indirect effects can severely hamper genetic progress [35, 36]. Moreover, a connection between the presence of indirect genetic effects and increased rates of inbreeding has been drawn [38]. In studies investigating the potential of genomic selection in honeybees, finite locus simulations that include maternal effects were implemented by Gupta, et al. [40, 41].

Strategies used to address the problem of uncertain paternity in several breeding schemes were first developed in [42] and [43]. By implementing computer simulations based on the infinitesimal model, Sullivan [44] showed a clear negative effect of uncertain paternity on breeding success. Cardoso and Tempelman [45] carried out simulation studies with uncertain paternity, including directly and maternally affected traits, and showed that they are equally affected by missing paternal information. Recently, a study conducted by Tonussi et al. [46] investigated the influences of unknown sires on genomic breeding value estimation based on a finite locus model. This study also revealed further implications of unknown paternity in the traditional BLUP setting, including the overestimation of genetic parameters.

The biology and current breeding schemes of honeybee show the aforementioned peculiarities to a heightened degree. They combine negatively correlated maternal (queen) and direct (worker) effects with an uncertain paternal descent and a strong need for the

maintenance of genetic diversity, which is threatened by inbreeding depression [47, 48]. Commercial traits, such as honey production or *Varroa* resistance, can be influenced maternally by the queen through her egg laying frequency or pheromone release, as well as directly by the workers (e.g., via nectar collection or hygienic behavior). Maternal and direct effects in honeybees differ slightly from their respective counterparts in mammals in two aspects.

- The direct effect is not attributed to an individual but to the collective of workers in a colony. It is, thus, often seen as their average effect [49].
- The maternal effect of a queen is not directed at the next generation of queens but at her worker group, an entity that is not directly involved in the selection process. Hence, selection for maternal effects does not suffer from the inaccessibility of information in the latest generation, which leads to reduced selection potential in maternally affected traits in other species [50].

Nevertheless, the notion of maternal and direct effects in honeybees resembles corresponding concepts in other farm animals very closely and are regularly seen as their equivalents [49, 51, 52]. This makes the honeybee an ideal model species to quantify the influence of previously unexamined factors.
Moreover, there is a specific current reason to investigate bee breeding. Because of a lack of selection, small honeybee races in Europe get increasingly replaced by two selected subspecies [53]. New breeding programs will soon be set up for these endangered races, and new performance testing protocols are being developed [54–57]. Therefore, at this point, sustainable long-term breeding strategies are needed.
After the establishment of a BLUP-based breeding value estimation for the honeybee [52, 58], a new species-specific methodology was developed [49, 59]. Primary simulation studies have been carried out using either the infinitesimal model [49] or finite locus models [40, 41]. In this work, we will explain the methodological concept, which is also transferable to other species, and establish simulation procedures for honeybee breeding schemes. We compare the long-term behavior of Monte Carlo simulations based on infinitesimal and finite locus models to facilitate reliable model choices for upcoming population-specific studies.

3.2 Methods

3.2.1 General assumptions

Honeybee colonies were modelled as consisting of a single queen and her offspring (a group of non-reproducing worker bees). In addition to the diploid queens and workers, haploid drones were individually modelled. After being created, young queens immediately mated with 12 drones each. Afterward, the genetic information of the queen and the 12 drones was used to create a worker group and daughter queens. Drones received their genetic information from their respective dam queens alone.
In accordance with common breeding practices (see [55] and [60] for more detailed explanations), drone producing queens (DPQ) and potential dams of the queens in the next generation (breeding queens, BQ) formed two mutually exclusive groups. A collection of eight DPQ formed a so-called mating station. The 12 drones, which an individual queen mated with, always came from one single mating station. The DPQ on a mating station were simulated to share a common dam BQ. In honeybee breeding theory, such mating stations are often seen as an analogue to sires in other farm animals [49, 52]. In situations where this analogy applies, we therefore refer to them as *pseudo sires*. The high maintenance effort of secure mating stations in reality leads to relatively small numbers of pseudo sires in honeybee breeding schemes. Our simulations covered the time period of 100 years. We simulated a small population in which 300 BQ and four pseudo sires were created each year, as well as a larger population with 1000 BQ and 10 pseudo sires. Each simulated year was generally characterized by the following events, which will be further discussed in detail:

- Queen production, including
  - Dam selection
  - Inheritance of true breeding values
- Queen mating
- Colony production and performance tests
- Breeding value estimation for the next year

The last two events occurred only in the second and later years.

The main simulations were implemented in C, while the creation of initial genetic distribution and the statistical analysis of the results were written in R [61]. The source code of the program as well as the output of the simulations and a script for the statistical analyses are stored in Dryad [62].

Dam selection

In each of the first two years, 300 (resp. 1000 in the larger population) BQ were created without specified parents. These base population BQ were assumed to be unrelated. Similarly, in each of the first three years, four (resp. 10) pseudo sires were created without specified parents. Every pseudo sire comprised eight DPQ. No relationships among DPQ or between DPQ and BQ were assumed in the first three years. Beginning in the third year, two-year-old BQ were available, and their colonies had estimated breeding values. With the breeding value estimation following the theory developed in [49], queens and their worker groups had direct and maternal estimated breeding values, where the sum of both breeding values of the worker group constituted the selection criterion (SC) (see [49, 63] for a detailed motivation of the SC). By truncation selection based on the SC, 60 (resp. 200) of the two-year-old BQ were chosen to serve as dams for the next generation of BQ. Each of the selected dams produced five new breeding queens. In the fourth year and each year thereafter, four (resp. 10) three-year-old BQ were selected as dams of the next generation of DPQ. The BQ were chosen by truncation selection based on the SC, and each of them mothered the setup of one mating station (see also Figure 3.1 for an overview of the breeding scheme).

3.2.2 Inheritance of true breeding values

The genetics of the bees were simulated according to three different models: (i) a finite locus model with 200 unlinked loci, (ii) a finite locus model with 400 unlinked loci, and (iii) the infinitesimal model. We will refer to the three models as FL200, FL400, and INF, respectively. For the simulations, we decided on a normally distributed trait with an initial direct (worker) additive genetic variance of $\sigma^2_{A,d} = 2$, an initial maternal (queen) additive genetic variance of $\sigma^2_{A,m} = 1$, and a residual effect with variance $\sigma^2_E = 1$. Two sets of simulations were run with different negative correlations between maternal and direct effects. In the first case, we chose a moderate negative covariance of $\sigma_{A,md} = -0.25$, and in the second case, we chose a stronger negative covariance of $\sigma_{A,md} = -0.75$. These

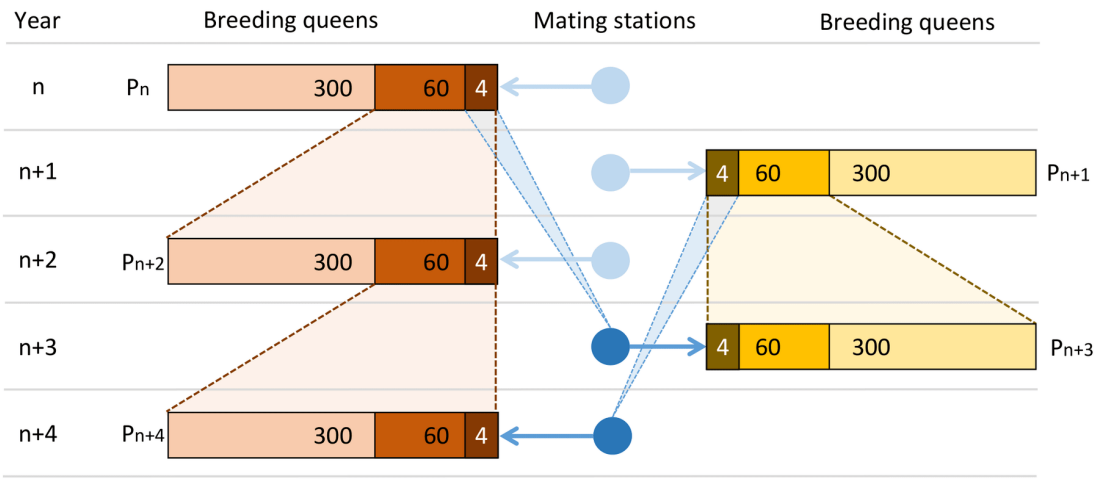

Figure 3.1: **Breeding scheme of the small population.** In each year, there are 300 new breeding queens (BQ), of which the best 60 produce the next generation of BQ (generation interval: two years). Each of the four best BQ produce a pseudo sire each year (generation interval: three years). For the larger population, the numbers (300, 60, 4) have to be replaced by (1000, 200, 10).

numbers reflect initial maternal effect heritabilities of $h_m^2 = 0.42$, resp. $h_m^2 = 0.53$, and direct effect heritabilities of $h_d^2 = 0.27$, resp. $h_d^2 = 0.34$, with genetic correlations of $r_{md} = -0.18$, resp. $r_{md} = -0.53$. These relations are in broad accordance with the breeding parameters determined for several traits by various studies [47, 63–65]. We note that as described in [65], the direct effect heritability h_d^2 measures the amount of phenotypic variance that can be attributed to the worker groups. It does not, however, reflect the scope for selection response when selecting virgin queens according to the theory developed in [66]. No dominance or epistatic effects were simulated.
We refer to the matrix of initial genetic parameters as

$$\mathbf{\Sigma}_A = \begin{pmatrix} \sigma^2_{A,d} & \sigma_{A,md} \\ \sigma_{A,md} & \sigma^2_{A,m} \end{pmatrix}. \tag{3.1}$$

The matrix $\mathbf{\Sigma}_A$ fulfilled three purposes in the simulations.

1. It defined the variance structure of breeding values in the base population.

2. It described the assumed genetic covariances in the BLUP breeding value estimation. ($\mathbf{\Sigma}_A$ was not updated for this purpose during the simulation, although the genetic parameters shifted due to drift and selection in the finite locus models)

3. It described the variance of Mendelian sampling in the inheritance of breeding values in the infinitesimal model. Also, for this purpose, $\mathbf{\Sigma}_A$ remained constant up to corrections for parental inbreeding during the simulation because selection does not affect the variance of Mendelian samplings in the infinitesimal model [67, 68].

We further refer to the distinct combinations of the genetic model (FL200, FL400, and INF), genetic correlations ($r_{md} = -0.18$ and $r_{md} = -0.53$), and population sizes (300 BQ and 1000 BQ per year) by adding the genetic correlations as subscripts and the population sizes as superscripts to the genetic model (see Table 3.1).

population size	genetic correl.	fin. locus, 200 loci	fin. locus, 400 loci	infinitesimal
300 BQ per year	$r_{md} = -0.18$	$\text{FL200}_{\text{-0.18}}^{\text{300 BQ}}$	$\text{FL400}_{\text{-0.18}}^{\text{300 BQ}}$	$\text{INF}_{\text{-0.18}}^{\text{300 BQ}}$
300 BQ per year	$r_{md} = -0.53$	$\text{FL200}_{\text{-0.53}}^{\text{300 BQ}}$	$\text{FL400}_{\text{-0.53}}^{\text{300 BQ}}$	$\text{INF}_{\text{-0.53}}^{\text{300 BQ}}$
1000 BQ per year	$r_{md} = -0.18$	$\text{FL200}_{\text{-0.18}}^{\text{1000 BQ}}$	$\text{FL400}_{\text{-0.18}}^{\text{1000 BQ}}$	$\text{INF}_{\text{-0.18}}^{\text{1000 BQ}}$
1000 BQ per year	$r_{md} = -0.53$	$\text{FL200}_{\text{-0.53}}^{\text{1000 BQ}}$	$\text{FL400}_{\text{-0.53}}^{\text{1000 BQ}}$	$\text{INF}_{\text{-0.53}}^{\text{1000 BQ}}$

Table 3.1: **Simulation settings.** Overview of the 12 different simulation settings with respect to the genetic model, population size, and correlation between the queen and worker effect.

Finite Locus Model

In the finite locus models, we assumed the trait to be genetically determined by 200 (FL200), respectively 400 (FL400), unlinked biallelic loci, each possessing a queen and a worker effect. In accordance with [67], we decided to use a U-shaped $\beta(0.5,0.5)$-distribution for the allele frequencies in the base population. The distribution of the allele effects is usually assumed to be heavy-tailed. Popular distributions for modelling such effects are gamma and exponential or Laplace distributions [69, 70], while [71] argues for a mixture of distributions. In [15], a mixture between a Laplace and a normal distribution with weights of 0.95 and 0.05 was utilized to describe the effects of the QTL. As this assumption allows for a straightforward generalization to the multivariate case, we also chose a distribution of QTL effects that was dominated by a Laplace distribution.

The genetic setup for the base population was created as follows:
Let a_1 and a_2 be the two possible alleles at a locus with frequencies p_1 and p_2, respectively. Let n be the total number of loci. Then the allele effects $\mathbf{E}_1 = \begin{pmatrix} E_1^d \\ E_1^m \end{pmatrix}$ and $\mathbf{E}_2 = \begin{pmatrix} E_2^d \\ E_2^m \end{pmatrix}$ of alleles a_1 and a_2 were determined in the following steps:

- Generate a random vector $\mathbf{e}$ following a distribution

$$0.95 \cdot \mathcal{L}(\mathbf{0}, \mathbf{\Sigma}_A) + 0.05 \cdot \mathcal{N}(\mathbf{0}, \mathbf{\Sigma}_A), \tag{3.2}$$

  where $\mathcal{L}$ and $\mathcal{N}$ denote the multivariate Laplace and normal distribution, respectively.

- Obtain preliminary allele effects

$$\tilde{\mathbf{E}}_1 = \frac{p_2 - p_1 + 1}{n}\mathbf{e}, \qquad \tilde{\mathbf{E}}_2 = \frac{p_2 - p_1 - 1}{n}\mathbf{e}. \tag{3.3}$$

By this transformation, the expected value for the allele effect at each locus is zero, and the expected additive genetic variance generated by all loci is $\mathbf{\Sigma}_A$. In a concrete realization, however, the obtained additive genetic variance $\tilde{\mathbf{\Sigma}}_A$ generally differs from $\mathbf{\Sigma}_A$. Therefore, after the preliminary allele effects were calculated for all loci, we applied a further post-correction step to obtain the allele effects and ensure that the additive genetic variance over all loci is $\mathbf{\Sigma}_A$.

- Calculate $\mathbf{E}_1$ and $\mathbf{E}_2$ as

$$\mathbf{E}_{1,2} = \mathbf{\Sigma}_A^{\frac{1}{2}} \tilde{\mathbf{\Sigma}}_A^{-\frac{1}{2}} \tilde{\mathbf{E}}_{1,2}. \tag{3.4}$$

The script for the creation of these possible alleles at the QTL was written in R [61] using the packages MASS [72] and L1pack [73] to create multivariate normal and Laplace distributed vectors, respectively [62]. Once the setup of possible alleles was generated, the queens of the base population were each equipped with two alleles at each locus, where the alleles were randomly drawn based on the specified frequencies. Queens of later generations inherited their genes by receiving one of the two alleles of the dam and the single allele of the sire drone at each gene locus. Drones inherited one of the two alleles of the dam at random. No mutations were modelled in the inheritance of alleles. For an individual queen, its true breeding value (TBV) was calculated as the sum of the

allele effects of its whole genome. To obtain comparable values for the TBV of haploid drones and diploid queens, the true breeding value of a drone was defined as twice the sum of the allele effects of its genome. This matches the interpretation of drones as diploid but homozygous at each locus as described in [74]. The worker group W of a queen Q received as its breeding value the mean value of Q's TBV and the average TBV of the drones that Q mated with, as shown in the following equation:

$$\mathbf{TBV}_W = \frac{1}{2}\left(\mathbf{TBV}_Q + \overline{\mathbf{TBV}}_D\right). \tag{3.5}$$

Infinitesimal Model

In the infinitesimal model (INF), all queens from the base population received their true breeding values as realizations of a normally distributed random vector with expectation $(0,0)'$ and variance $\mathbf{\Sigma}_A$, as explained in [75]. The inheritance of true breeding values from parents to offspring followed the theory of [76] with necessary adaptations to the haploidy of drones. In particular, the inheritance of the TBV from a breeding queen B and a drone D to a daughter queen Q was realized as

$$\mathbf{TBV}_Q = \frac{1}{2}\left(\mathbf{TBV}_B + \mathbf{TBV}_D + \sqrt{1-F_B}\cdot\mathbf{\Phi}\right), \tag{3.6}$$

where the Mendelian sampling $\mathbf{\Phi}$ was the realization of a normally distributed random vector with expectation $(0,0)'$, and variance $\mathbf{\Sigma}_A$. F_B denotes the inbreeding coefficient of B. *In vivo*, exact estimations of relationships and inbreeding coefficients are difficult to obtain because the relatedness of two offspring of the same dam can vary between 0.25 and 0.75 [52]. However, *in silico*, drones were simulated individually, so we could keep track of the exact relationships between siblings and, consequently, between all individuals. The calculation of relationships was performed as stated in [77]. Therefore, at this point, the simulations did not need to rely on approximated calculations of F_B as described in [52] or [49, 59]. The inheritance from a DPQ Q to a drone D was realized as

$$\mathbf{TBV}_D = \mathbf{TBV}_Q + \sqrt{1-F_Q}\cdot\mathbf{\Phi}, \tag{3.7}$$

where again the Mendelian sampling $\mathbf{\Phi}$ was the realization of a normally distributed random vector with expectation $(0,0)'$ and variance $\mathbf{\Sigma}_A$. The worker group W of a queen Q received its breeding value as in the finite locus models according to Equation 3.5.

3.2.3 Queen mating

When all queens of a year were created, the BQ were assorted to the pseudo sires. BQ with the same dam were sent to the same mating station. The distribution of the sister groups to the pseudo sires was random. When a queen was assigned to a pseudo sire, 12 drones were produced, where the dam of each drone was chosen at random among the 8 DPQ of the mating station. The data of the drones (alleles, in the case of the finite locus models, and TBV, in the case of the infinitesimal model) were henceforth stored together with the queen data.

3.2.4 Colony production and performance tests

After the mating of the newly-born BQ, the one-year-old BQ were assigned a colony. The colony received true breeding values according to Equation 3.5. The simulated performance of a colony was calculated as the sum of the true maternal breeding value of the queen ($\mathbf{TBV}_Q^m$), the true direct breeding value of the worker group ($\mathbf{TBV}_W^d$), and a random residual effect being the realization of a normally distributed random variable with a mean of zero and variance of σ_E^2. As the sum $\mathbf{TBV}_Q^m + \mathbf{TBV}_W^d$ determines the genetic share of the outcome of the performance test, the main aim of the selection is to maximize this value over time. Therefore, we refer to this sum as the performance criterion (PC).
Subsequently, a BLUP breeding value estimation was carried out using the BLUPF90 software [78] with the relationships between BQ and colonies calculated as described in [59]. The breeding value estimation was based on the parameters $\sigma_E^2 = 1$ and $\mathbf{\Sigma}_A$ as specified in Equation 3.1. No parameter estimation was carried out within the simulations, and the BLUP parameters were not adjusted to represent genetic changes due to drift and selection in settings FL200 and FL400.

3.2.5 Repetitions

Simulations using the INF model were repeated 100 times to obtain reliable results. In the simulations with a finite number of loci, we created 10 sets of allele frequencies and allele effects for each of the finite locus settings. With each of these sets, simulations for both population sizes were repeated 100 times.
A posteriori, we did an investigation on the repetitions needed for future simulations based on the variance of the outcomes within the respective repetitions. Following [79], we estimated the number of needed repetitions of the simulations from the variances in

the results. If the standard deviation of the result of the repetitions is s, and the tolerated error in the estimation for the mean value is e, then, with a minimum repetition number of

$$N_{\min} = \left(\frac{s \cdot z_{1-\frac{\alpha}{2}}}{e} \right)^2, \tag{3.8}$$

there is a security level of $1-\alpha$ that the calculated mean value differs from the true expectation by less than e. Here, z refers to the quantiles of the standard normal distribution. As the outcomes of the simulations depend on the chosen model and deviations between simulation results, and *in vivo* observations due to model inaccuracy are possible, we decided to allow for an error rate of up to 10%. We further investigated how many repetitions are needed to fall below this error rate with a confidence level of 99%.

3.3 Results

3.3.1 Genetic gain

Table 3.2 summarizes the average accumulated genetic gain in the breeding colonies. The genetic gain for the performance criterion (PC) after 100 years ranged from 14.90 units in model $\text{FL200}^{300\text{ BQ}}_{\text{-0.53}}$ to 41.37 units in model $\text{INF}^{1000\text{ BQ}}_{\text{-0.18}}$. In all settings, the genetic value for the direct effect increased more than that of the maternal effect. This remained the case when we corrected for the different genetic standard deviations of maternal and direct effects. The ratio between genetic gain for direct and maternal effects after 100 years ranged from 1.58 ($\text{FL400}^{300\text{ BQ}}_{\text{-0.18}}$) to 2.81 ($\text{FL400}^{1000\text{ BQ}}_{\text{-0.53}}$) before correction and 1.12 ($\text{FL400}^{300\text{ BQ}}_{\text{-0.18}}$) to 1.99 ($\text{FL400}^{1000\text{ BQ}}_{\text{-0.53}}$) after correction.

Throughout all settings, the genetic values for the worker groups were, on average, slightly higher than those for the corresponding queens. The models with strong negative correlations showed a lower genetic gain than the corresponding model with a low negative correlation. This remained true when we corrected the genetic gain by the different genetic standard deviations. A larger population size had a slight positive effect on genetic gain. The ratio between the genetic gains for different population sizes in the PC after 100 years ranged between 1.10 ($\text{FL200}_{\text{-0.18}}$) and 1.17 ($\text{INF}_{\text{-0.53}}$). In the long-term, model INF always predicted the highest genetic gain, followed by FL400 and FL200, in this order. The ratio between genetic gain in the PC under model INF and model FL200 ranged between 1.62 (300 BQ, $r_{md} = -0.53$), and 1.74 (1000 BQ, $r_{md} = -0.18$) after 100 years. After 20 years, this ratio only ranged between 1.013 (300 BQ, $r_{md} = -0.18$) and 1.038

r_{md}	-0.53						-0.18					
model	FL200		FL400		INF		FL200		FL400		INF	
BQ per year	300	1000	300	1000	300	1000	300	1000	300	1000	300	1000
	before correction for different genetic standard deviations											
	after 20 years											
maternal effects	1.88	1.77	1.85	1.73	1.93	1.74	2.99	3.03	3.01	3.05	2.99	3.10
direct effects	3.18	3.73	3.31	3.91	3.33	3.96	4.67	5.18	4.67	5.18	4.76	5.30
perf. crit.	5.08	5.54	5.18	5.69	5.26	5.75	7.69	8.27	7.74	8.30	7.80	8.46
	after 100 years											
maternal effects	5.27	4.96	5.57	5.22	8.09	7.94	8.28	8.57	9.99	10.52	13.67	14.71
direct effects	9.62	11.73	11.84	14.66	15.96	20.14	13.37	15.26	15.78	18.22	22.54	26.60
perf. crit.	14.90	16.70	17.40	19.88	24.08	28.14	21.65	23.83	25.79	28.76	36.23	41.37
	after correction for different genetic standard deviations											
	after 20 years											
maternal effects	1.88	1.77	1.85	1.73	1.93	1.74	2.99	3.03	3.01	3.05	2.99	3.10
direct effects	2.25	2.64	2.34	2.77	2.36	2.80	3.30	3.66	3.30	3.66	3.36	3.75
perf. crit.	3.38	3.69	3.45	3.80	3.51	3.83	4.64	4.99	4.67	5.01	4.70	5.10
	after 100 years											
maternal effects	5.27	4.96	5.57	5.22	8.09	7.94	8.28	8.57	9.99	10.52	13.67	14.71
direct effects	6.80	8.29	8.37	10.36	11.29	14.24	9.45	10.79	11.16	12.89	15.94	18.81
perf. crit.	9.93	11.13	11.60	13.25	16.05	18.76	13.05	14.37	15.55	17.34	21.85	24.95

Table 3.2: **Genetic Gain.** The average genetic gain in the different models after 20 and 100 years. Maternal and direct effects were taken from the BQ of that year; PC denotes the performance criterion. In the lower part of the table, values are divided by the the respective initial genetic standard deviations to improve their comparability.

(1000 BQ, $r_{md} = -0.53$). Genetic gain in model INF showed an almost linear increase, whereas models FL200 and FL400 showed clearly sublinear behavior after 20 to 25 years (see Figure 3.2).

3.3.2 Genetic variance and inbreeding

The development of the mean inbreeding coefficients of the breeding queens depended mainly on the population size. After 100 years, the average inbreeding coefficient in the small populations was between 0.37 (model $\mathrm{FL200}_{-0.18}^{300\ \mathrm{BQ}}$) and 0.43 (model $\mathrm{INF}_{-0.53}^{300\ \mathrm{BQ}}$). The corresponding values in the larger populations were 0.24 ($\mathrm{FL200}_{-0.18}^{300\ \mathrm{BQ}}$) and 0.30 ($\mathrm{INF}_{-0.53}^{1000\ \mathrm{BQ}}$). This means there were yearly increases of about $\Delta F = 0.004$ and $\Delta F = 0.003$, respectively. However, the development of genetic diversity in the population de-

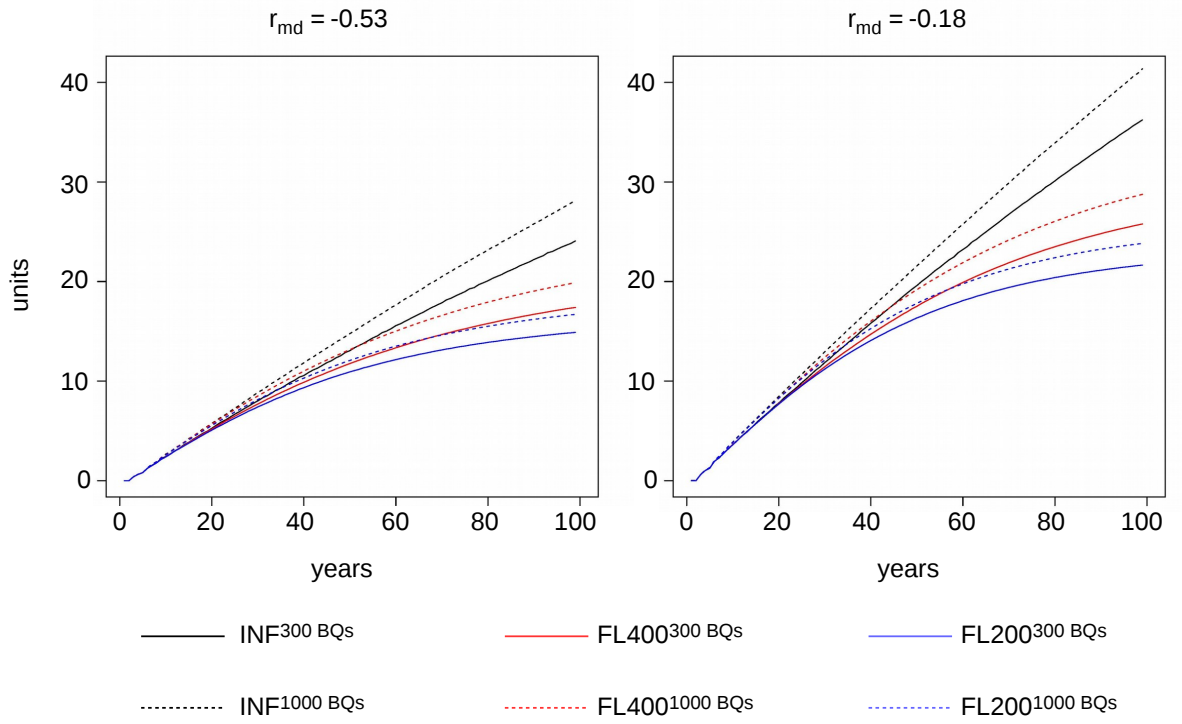

Figure 3.2: **Genetic gain in the performance criterion.** Development of the genetic gain in the performance criterion (PC) over the course of 100 years in the models INF (black), FL400 (red), and FL200 (blue) for population sizes consisting of 300 breeding queens (solid lines) and 1000 breeding queens (dashed lines), respectively. Results are shown for a strong negative correlation between maternal and direct effects, $r_{md} = -0.53$ (left hand side), and a weak negative correlation, $r_{md} = -0.18$ (right hand side).

pended much more on the underlying genetic model than on the population size. All simulations showed an initial decrease in genetic variance due to the Bulmer effect [68], but after 100 years, only the finite locus models showed a tremendous decay in genetic variance (see Figure 3.3). After 100 years, the variances in the PC in the INF model only went down to 52% for the small population and to between 64% and 67% for the larger population. The corresponding values for the finite locus models ranged from 8.3% ($FL200_{-0.18}^{300\ BQ}$) to 24.4% ($FL400_{-0.53}^{1000\ BQ}$). The ratio between genetic variance in the PC under the model INF and model FL200 ranged between 3.88 (300 BQ, $r_{md} = -0.53$) and 6.58 (1000 BQ, $r_{md} = -0.18$) after 100 years. After 20 years, this ratio only ranged between 1.07 (300 BQ, $r_{md} = -0.53$) and 1.13 (300 BQ, $r_{md} = -0.18$).

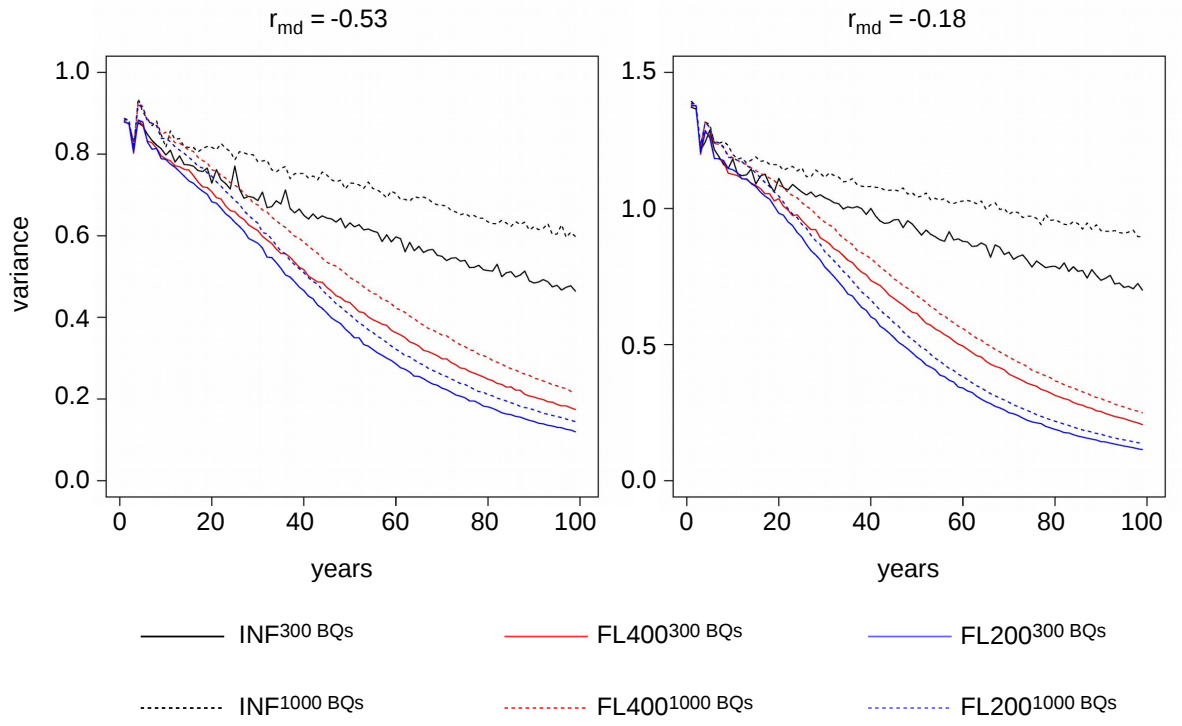

Figure 3.3: **Additive genetic variance in the performance criterion.** Development of the additive genetic variance in the performance criterion (PC) over the course of 100 years in the models INF (black), FL400 (red), and FL200 (blue) for population sizes consisting of 300 breeding queens (solid lines) and 1000 breeding queens (dashed lines), respectively. Results are shown for a strong negative correlation between maternal and direct effects $r_{md} = -0.53$, (left hand side) and a weak negative correlation $r_{md} = -0.18$ (right hand side).

3.3.3 Variance in the results

Since all simulations were Monte Carlo simulations, variance could be observed in the results. In models FL200 and FL400, there were two sources of variance. One source was the 10 different distributions of allele effects, and the other source was the 100 repetitions that were carried out with each distribution. In model INF, only the latter source of variance was present. The standard deviations in the results with a fixed distribution of allele effects after 20 and 100 years are shown in Table 3.3. When we compare these standard deviations with the realized mean values in the genetic gain, we find a relatively high standard deviation of up to 25% in the maternal true breeding values, whereas the standard deviation in the PC is relatively low (under 10%). When we consider the

standard deviations in the genetic variance, we obtain high percentages for the PC (31.1% in model $FL200^{300\ BQ}_{-0.53}$) and slightly lower percentages of around 20% for the maternal and direct true breeding values. In the models FL200 and FL400, the different distributions of allele effects mostly had only small influences of under 10% on genetic gain and variance.

r_{md}	-0.53						-0.18					
model	FL200		FL400		INF		FL200		FL400		INF	
BQ per year	300	1000	300	1000	300	1000	300	1000	300	1000	300	1000
	population mean											
	after 20 years											
maternal effects	0.466	0.347	0.441	0.346	0.446	0.326	0.404	0.299	0.394	0.294	0.407	0.333
direct effects	0.602	0.451	0.611	0.446	0.618	0.390	0.562	0.389	0.557	0.401	0.533	0.392
perf. crit.	0.458	0.346	0.489	0.358	0.547	0.342	0.547	0.375	0.531	0.381	0.598	0.397
	after 100 years											
maternal effects	0.776	0.596	0.829	0.681	0.901	0.700	0.745	0.595	0.734	0.600	0.879	0.711
direct effects	1.001	0.827	1.064	0.913	1.178	0.889	0.980	0.813	0.989	0.820	1.103	0.812
perf. crit.	0.727	0.583	0.774	0.671	0.884	0.658	0.966	0.830	0.961	0.780	0.844	0.701
	population variance											
	after 20 years											
maternal effects	0.126	0.096	0.118	0.090	0.113	0.102	0.123	0.086	0.111	0.078	0.110	0.074
direct effects	0.222	0.156	0.215	0.150	0.196	0.139	0.214	0.148	0.199	0.138	0.190	0.119
perf. crit.	0.129	0.097	0.125	0.092	0.125	0.097	0.151	0.110	0.151	0.110	0.135	0.096
	after 100 years											
maternal effects	0.066	0.058	0.066	0.060	0.067	0.055	0.052	0.047	0.051	0.048	0.071	0.059
direct effects	0.110	0.082	0.113	0.094	0.119	0.093	0.085	0.072	0.085	0.069	0.148	0.092
perf. crit.	0.037	0.032	0.049	0.042	0.091	0.062	0.033	0.029	0.045	0.040	0.089	0.077

Table 3.3: **Genetic gain.** Standard deviations in the outcomes of the simulations with a fixed setting. Maternal and direct effect were taken among the BQ of that year. PC denotes the performance criterion.

3.3.4 Bias in estimated breeding values

When we compared the estimated BLUP breeding values of individuals with their true breeding values, we saw no bias in the simulations based on model INF. The estimated breeding values in the settings FL200 and FL400, however, developed an increasing bias over time (see Figure 3.4). After 100 years of selection, the ratios between estimated and true breeding values for the selection criterion SC ranged from 1.27 in setting $FL400^{300\ BQ}_{-0.18}$ to 1.45 in setting $FL200^{1000\ BQ}_{-0.53}$.

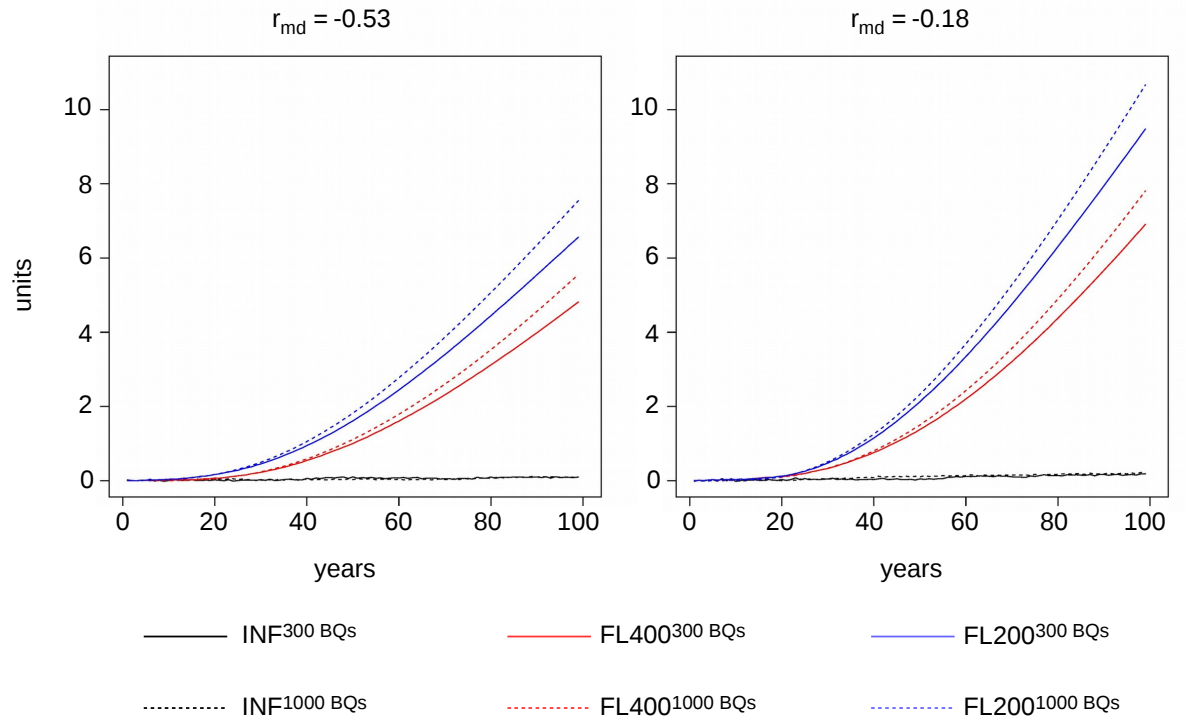

Figure 3.4: **Bias of estimated breeding values.** Development of the bias of estimated breeding values in the selection criterion (SC) over the course of 100 years in the models INF (black), FL400 (red), and FL200 (blue) for population sizes consisting of 300 breeding queens (solid lines) and 1000 breeding queens (dashed lines), respectively. Results are shown for a strong negative correlation between maternal and direct effects, $r_{md} = -0.53$, (left hand side) and a weak negative correlation, $r_{md} = -0.18$ (right hand side).

3.4 Discussion

3.4.1 Model selection

In the finite locus model, all loci were assumed to be potentially pleiotropic, whereas, in reality, many loci will only contribute to either direct or maternal effects. Works by Guo et al. [29] and Gupta et al. [41] consequently proposed models where only a part of the loci was pleiotrophic. In contrast, [29] and [41] proposed models where many of the loci contribute to either the direct or maternal effects. As a tradeoff, however, [29] had to assume that all pleiotrophic loci have the exact same contribution on every trait, which seams unrealistic. In [41], the genetic covariance between direct and maternal effects

could not be defined *a priori* but only determined *a posteriori* once a base population was created.

Furthermore, neither of the approaches in [29, 41] allow for a straightforward generalization for arbitrary multiple trait models. Our choice of the multivariate Laplace distribution was made because it allows for natural generalizations to multiple trait models and is in line with common one-dimensional assumptions, such as the QTL effects following a heavy-tailed distribution [15]. The strong negative correlation between direct and maternal effects observed in the honeybee [47, 63–65] indicates that in this species, many loci are indeed pleiotrophic or loci with direct and maternal effects that are closely linked with each other. An *a posteriori* investigation of the simulated allele effects showed that although all loci were potentially pleiotrophic, about a fourth of the loci showed practically only one effect, with minor differences in the different genetic setups. See Appendix S1 for details.

Nevertheless, detailed investigations with genetic data resembling reality as closely as possible are desirable, especially when more honeybee-specific information is available in the future due to the introduction of high definition SNP-chips for the honeybee [80]. Based on the explanations given above, we expect that gene loci will prove more heterogeneous than assumed in our models. Therefore, the assumptions of the infinitesimal model will be violated to an even greater extent. Hence, it is to be expected that the results will show an even greater deviation. With more knowledge about the properties of specific traits, it will also be possible to further investigate the interplay of different traits with their direct and maternal expressions, as well as nonadditive effects.

To our knowledge, there are no suitable estimates for the number of loci that control the various traits in honeybee breeding. In other species, the estimated number of QTLs responsible for a trait has increased drastically within the last two decades, to numbers above 2000 [81]. Hence, the numbers of 200 and 400 loci that we chose are rather conservative. They are, however, in line with the findings and assumptions of [70] for a swine population, as well as [41] for the honeybee.

The linkage of gene loci has an influence on the accuracy of BLUP-based breeding value estimation [82]. This effect is due to the fact that linkage shrinks the effective number of loci responsible for a trait [83, 84]. As all loci in our simulations were considered unlinked, this also justifies the relatively small number of QTL. However, linkage may diminish the rate of drift and, therefore, lead to a smaller bias in breeding value estimation. We conducted a small study based on setting $\mathrm{FL400}^{300\ \mathrm{BQ}}_{-0.18}$ in which we included linkage

between the loci based on the honeybee genome but found only minor differences from the simulations without linkage. Details on this smaller-scale study are given in Appendix S2.

While linkage does not play a major role in our simulation, it must necessarily be included in simulation studies that rely on genomic selection strategies [40, 41]. If such simulations are carried out for the honeybee, one should account for the high recombination rate in this species [85–87]. Linkage effects and the high recombination rate will also play a role if future simulations focus on the sex-determining locus [88].

3.4.2 Comparison of the models

Direct and maternal genetic gain

The overall findings in genetic development with the close-to-linear genetic gain in model INF and sublinear behavior in the finite locus models is in line with existing simulation studies [19, 89].

Linksvayer and Wade [90, 91] showed that under natural selection, fitness traits that depend on the genetic properties of the worker group have a reduced selection potential compared to fitness traits that depend on the genetic properties of a queen. However, as by the definition of the selection criterion only the estimated (direct and maternal) breeding values of the worker group are taken into consideration for the breeding decisions, the approaches of [90, 91] cannot explain different developments of direct and maternal effects. The negative correlation between direct and maternal effects has been long known to complicate breeding decisions and, when it is not taken care of correctly, it can even cause the selection to run in the opposite direction of what was intended [92]. The observation that the direct effects underwent a larger genetic gain than the maternal effects, also relative to the respective additive variances, can be explained as follows. As the direct effects had a higher genetic variance than the maternal effects and both effects are negatively correlated, in many alleles with adverse direct and maternal effects, the direct outweighed the maternal component. At these loci, selection was therefore directed actively against the maternal effects which caused the different developments. Another factor that influences the genetic development is the accuracy of breeding value estimation. In our simulations, the correlation between true and estimated breeding values of the worker groups were slightly higher for the maternal effects (between 0.54 and 0.65, depending on the simulation setting) than for the direct effects (between 0.46

and 0.57). These differences were too small to impact the preference for direct effects significantly. However, with equal additive genetic variances of direct and maternal effects, the improved estimation accuracy will cause a stronger selection for maternal effects. A small simulation study with varying additive genetic variances for the direct and maternal effects confirms these theoretical considerations. It is included in Appendix S3. Likewise, the simulations in [35, 36] also assumed higher direct than maternal genetic variances and predict a main selection focus on the direct effects. These studies even prognosticate a negative selection of the maternal effects when an even stronger negative correlation with the direct effects occurs. Further simulation studies that shed more light on the development and consequences of the negative correlation between direct and maternal effects under selection and genetic drift are highly desirable.

Development of genetic variance

When it comes to genetic variance, the observed drastic decay in the finite locus models matches the findings of [18]. We ascribe the lower genetic gain and variance in the finite locus models mainly to the inhomogeneity of the allele effects. Alleles with a higher impact on the selection trait have the highest contribution to the genetic variance but are also the genes that are mainly selected for. Therefore, in a finite locus model where few loci contribute to a large amount of the genetic variance, it is precisely the favorable alleles at these loci that have the highest tendency to become fixed within the population [93]. The larger population could preserve more genetic variance over time and could, therefore, maintain a more sustainable genetic gain. This is in line with earlier simulation studies [19, 94]. However, different population sizes showed only minor differences in the development of genetic variance. We conclude that the decay of genetic variability is mainly caused by the selection intensity and that genetic drift and the accumulation of inbreeding play only a smaller role as these are clearly influenced by the effective population size. We expect that lowering the ratio of BQ to pseudo sires could improve the preservation of genetic variance as this would immensely weaken the selection intensity on the paternal side. Such a measure is likely to decrease genetic gain in the short-term, but in the long-term, it may be profitable [94, 95]. Differences in the outcomes of the three models suggest that in simulations exceeding 20 years (i.e., 10 generations on the female and seven generations on the male side), the genetic setup has to be chosen carefully.

Finite locus models, as we implemented them, fail to explain the phenomenon of 'missing heritability' [96]. This term describes the observation that the additive genetic variance in

several quantitative traits can only be partially explained by the QTL associated with this trait. Consequently, [97] suggests relying on the infinitesimal model despite its biologically incorrect assumptions. In [97], the author provides examples of long-term selection in poultry that showed a linear development over a span of 50 generations and beyond. In contrast, [98] showed a substantial loss in genetic variance in a selection experiment for low mating activity in Japanese quail, and [99] succeeded in explaining most of the additive genetic variance in complex traits in cattle by genetic markers. Without further knowledge about the actual genetic architecture of the simulated trait, one will always bear the risk that long-term simulation studies based on the infinitesimal model are likely to give overly optimistic predictions on the conservation of genetic variance. However, predictions based on finite locus models might be too pessimistic. An interesting field of research appears to be the question of how non-additive genetic effects (dominance, epistasis) can support the maintenance of additive genetic variance in the finite locus model [15].

Variance in the results

One possible explanation for the relatively high variance in the results for the average maternal breeding value of the population is that selection focused more on the direct trait component, and the selection of the maternal part of the trait was, therefore, less stringent. However, further investigation of this finding is necessary.
The required repetitions for secure results that we calculated via Equation 3.8 can be found in Table 3.4. They are all well below 100, so our simulation setup was adequate. The different realized distributions of allele effects within a finite locus model showed only minor effects on the outcomes. We conclude that a single realization will suffice for many purposes and that more diverse results are to be expected with the introduction of more distributions rather than more realizations of the same distribution [12, 18].

Estimated breeding values

In the INF model, it was to be expected that no bias would occur in the estimated breeding values since BLUP is an unbiased estimator for breeding values in this model [100]. In finite locus models, however, a shift occurs in allele frequencies, leading to a reduction of heritabilities. As BLUP was run relying on the initial genetic parameters throughout the simulations, the estimated breeding values were positively biased in these cases. The effects of the biased estimates for heritabilities of the genetic response have been shown to

r_{md}	-0.53						-0.18					
model	FL200		FL400		INF		FL200		FL400		INF	
BQ per year	300	1000	300	1000	300	1000	300	1000	300	1000	300	1000
	population mean											
	after 20 years											
maternal effects	42	26	38	27	36	24	13	7	12	7	13	8
direct effects	24	10	23	9	23	7	10	4	10	5	9	4
perf. crit.	6	3	6	3	8	3	4	2	4	2	4	2
	after 100 years											
maternal effects	15	10	15	12	9	6	6	4	4	3	3	2
direct effects	8	4	6	3	4	2	4	3	3	2	2	1
perf. crit.	2	1	2	1	1	1	2	1	1	1	1	1
	population variance											
	after 20 years											
maternal effects	17	9	15	8	13	9	18	8	14	6	13	5
direct effects	15	7	12	6	10	5	14	7	12	6	10	4
perf. crit.	25	12	21	10	20	10	16	8	15	7	10	5
	after 100 years											
maternal effects	54	30	34	19	12	5	63	37	29	17	13	6
direct effects	57	28	32	17	10	4	59	38	27	14	14	4
perf. crit.	67	34	53	26	26	8	57	33	32	18	11	5

Table 3.4: **Required repetitions.** Numbers of repetitions required for a simulation to have a 99% confidence level that the deviation is less than 10%.

be small, while inbreeding would probably have increased even more drastically if BLUP was run with the correct parameters [101]. The simulations show the need in real life breeding to re-estimate genetic parameters regularly unless one explicitly wants to make use of the positive effects of biased parameter estimates on the development of inbreeding as described in [101].

3.4.3 Honeybees

Our methodological approach and the results are usable for all livestock species. In our opinion, considerations of sustainable long-term breeding concepts have not yet been adequately addressed. This applies, in particular, to the honeybee as the complementary

sex determination makes this species especially vulnerable to inbreeding depression [48]. Therefore, a strong focus on the maintenance of genetic diversity is mandatory. Moreover, one has to consider that the replacement of native subspecies by two selected races has reached a degree that leaves many subspecies threatened by extinction [53]. The EU project SmartBees [102] aims to establish breeding strategies for these subspecies, which are well adapted to their regional environmental conditions, to comply with the breeders' productivity requirements. With their main purpose being the conservation of subspecies, these breeding strategies must be sustainable despite small effective population sizes. The methods we developed here are a good foundation to address such questions.
Unfortunately, the chosen example with 300 colonies and four pseudo sires is representative for some subspecies, such as the Maltese honeybee *Apis mellifera ruttneri* [103] or the Sicilian honeybee *A. m. siciliana* [104]. They need breeding approaches that are substantially different from those of the large breeding population of *A. m. carnica* with 8000 colonies per year, which has shown a strong response to selection within the last two decades [105]. The simulations indicate that the chosen breeding schemes, which we initially saw as realistic, are too strict and will potentially harm any population of honeybees. For practical purposes, other breeding strategies with more pseudo sires or a reduction in the number of offspring within one family, according to [106], will have to be developed. A transfer of the Optimum Contribution Selection [107] to the haplo-diploid case also seems promising.

3.5 Conclusion

We conclude that future long-term simulation studies that design strategies in conservational animal breeding should focus on finite locus models rather than the infinitesimal model to minimize the risk of overly optimistic prognoses. Differences in the outcomes of simulations of type FL200 and FL400 indicate the need for more accurate information on the number and distribution of QTL. Of course, a simulation output of 100 years should always be seen as a reference timeframe as the breeding infrastructure is likely to undergo major changes in this time. Currently, the introduction of genomic selection for the honeybee is assumed to give rise to new breeding strategies [40, 41, 60]. For short-term simulation studies of less than 10 generations, all genetic models appear to be equally feasible.

3.6 Appendix S1: QTL effect analysis

For our simulation study we generated ten different distributions of allele frequencies and QTL effects for each of the eight settings $\mathrm{FL200}_{-0.18}^{300\ \mathrm{BQ}}$, $\mathrm{FL200}_{-0.53}^{300\ \mathrm{BQ}}$, $\mathrm{FL200}_{-0.18}^{1000\ \mathrm{BQ}}$, $\mathrm{FL200}_{-0.53}^{1000\ \mathrm{BQ}}$, $\mathrm{FL400}_{-0.18}^{300\ \mathrm{BQ}}$, $\mathrm{FL400}_{-0.53}^{300\ \mathrm{BQ}}$, $\mathrm{FL400}_{-0.18}^{1000\ \mathrm{BQ}}$, and $\mathrm{FL400}_{-0.53}^{1000\ \mathrm{BQ}}$. The maternal and direct QTL-effects followed a distribution that was dominated by a multivariate Laplace distribution. Therefore, all gene loci were potentially pleiotrophic regarding direct and maternal effects. In reality, however, there will probably be many loci, that only have influence on either the maternal or the direct effect. Therefore we wanted to investigate, how many of the simulated loci actually proved to be pleiotrophic.

For this purpose, we divided the loci into three categories regarding maternal effects and three categories regarding direct effect, leading to nine cross categories: A locus could have a definite effect, an unclear effect or practically no effect maternally and directly.

For the direct effect, the loci were categorized as follows: For a locus l, we let $E_{l,1}^d$, $E_{l,2}^d$ be the direct effects of the two possible alleles at this locus. Then the range of possible direct contribution of the locus l is

$$R_l^d := |E_{l,1}^d - E_{l,2}^d|.$$

Let n be the total number of loci and let the loci be numbered from 1 to n so that $R_i^d \leq R_j^d$ for $i < j$. Then

$$R^d := \sum_{l=1}^{n} |E_{l,1}^d - E_{l,2}^d|$$

describes the total range of possible true direct breeding values. Let n_1 be maximal such that

$$\sum_{l=1}^{n_1} |E_{l,1}^d - E_{l,2}^d| < 0.05 \cdot R,$$

then we considered the loci with the numbers 1 to n_1 to have practically no direct effect. Conversely, we considered the loci l with

$$|E_{l,1}^d - E_{l,2}^d| > \frac{1}{2n} \cdot R$$

to definitely have a direct effect. All other loci were considered to have an unclear direct effect. For the maternal effect we applied the same definitions. This allowed us to consider these loci as not pleiotrophic that definitely had a direct effect but practically no maternal

effect or vice versa. Table 3.5 gives a survey of the relative frequencies of alleles inside the nine cross categories.

r_{md}	number of loci			maternal effect		
				practically no	unclear	defititive
-0.18	200	direct effect	practically no	12.1%	3.45%	14.2%
			unclear	3.3%	1.0%	6.15%
			definitive	13.95%	5.9%	39.95%
	400	direct effect	practically no	12.3%	3.4%	13.28%
			unclear	3.45%	1.18%	4.93%
			definitive	13.43%	5.65%	43.15%
-0.53	200	direct effect	practically no	13.9%	3.4%	12.25%
			unclear	3.15%	0.9%	4.8%
			definitive	12.9%	5.55%	43.15%
	400	direct effect	practically no	14.18%	3.68%	12.08%
			unclear	3.3%	0.98%	4.55%
			definitive	12.33%	5.23%	43.7%

Table 3.5: **Allele frequencies.** Frequencies of alleles in different categories for direct and maternal effects.

With little deviances for the different models we found that only slightly more than 40% of the loci turned out clearly pleiotrophic. About 13% of the loci carried practically no effect at all, while 13% had only a maternal effect and another 13% had only a direct effect. For the rest of the loci it could not be clearly decided if they have a maternal or a direct effect.

3.7 Appendix S2: Linkage simulations

Our main simulation studies used unlinked loci in the finite locus models. In order to investigate if the assumption of unlinkedness caused a substantial bias to our results, we also performed a small scale study using linked loci. For this purpose, we generated a setup of possible alleles for 400 loci[2] with $r_{md} = -0.18$. We followed the descriptions of

[2]The published version wrongly speaks of 200 loci

the main article up to the calculation of the preliminary allele effects $\tilde{\mathbf{E}}_1$ and $\tilde{\mathbf{E}}_2$ according to Equation 3.3. Afterwards, we placed the 200 loci randomly on 12 chromosomes, with chromosome lengths and recombination probabilities as in [40]. Only then did we apply the correction of the additive genetic variance according to Equation 3.4. With this genetic setup, we performed 20 repetitions of simulations as in setting $\mathrm{FL400}^{300\ \mathrm{BQ}}_{-0.18}$. Table 3.6 compares the outcomes of this simulation setup with the original results for setting $\mathrm{FL400}^{300\ \mathrm{BQ}}_{-0.18}$.

	genetic gain					
	after 20 years			after 100 years		
	mat. eff.	dir. eff.	PC	mat. eff.	dir. eff.	PC
with linkage	3.35	4.82	8.29	10.39	16.84	27.23
without linkage	2.03	4.67	7.73	10.06	15.75	25.83
	genetic variance					
	after 20 years			after 100 years		
	mat. eff.	dir. eff.	PC	mat. eff.	dir. eff.	PC
with linkage	0.799	1.669	1.122	0.249	0.422	0.214
without linkage	0.787	1.537	1.036	0.246	0.427	0.206
	Bias of EBV					
	after 20 years			after 100 years		
	mat. eff.	dir. eff.	SC	mat. eff.	dir. eff.	SC
with linkage	-0.129	-0.021	-0.188	2.594	3.260	5.830
without linkage	0.045	0.094	2.714	4.181	6.904	11.059[3]

Table 3.6: **Comparison of simulations.** Comparisons of simulations in setting $\mathrm{FL400}^{300\ \mathrm{BQ}}_{-0.18}$, with and without linkage.

We did not find any qualitative differences in the genetic gain and the loss of genetic variance over time. The slower drift rate due to linkage caused a weaker bias in the estimated breeding values and thus to a more accurate breeding value estimation. This effect can explain the slightly higher total genetic gain in the model with linkage.

[3]Value missing in original publication.

3.8 Appendix S3: Selection strength on direct and maternal effects

Our main simulation studies investigated selection of a trait that was influenced maternally and directly. Hereby, the initial additive genetic variance of the direct effects, $\sigma^2_{A,d}$, was always twice as high as the corresponding value for the maternal effects, $\sigma^2_{A,m}$. We found that selection always focused primarily on the direct effects, also when we corrected for the different additive genetic variances. These results were in line with findings in [35, 36], where also a higher direct variance was assumed. In order to investigate the influence of different direct and maternal heritabilities on the selection focus, we conducted a small scale investigation based on the setting $\text{INF}^{300\ \text{BQ}}$. We let the initial maternal additive variance vary from 1.0 to 2.0 in steps of 0.1 and adjusted the direct additive variance accordingly, so that

$$\sigma^2_{A,m} + \sigma^2_{A,d} = 3$$

remained constant. With each choice of genetic parameters we conducted 20 simulations over the course of 50 years. Figure 3.5 shows the ratio between direct and maternal genetic gain after 50 years of selection for different rates of initial genetic variance.[4]

We found that with equal maternal and direct heritabilities, the selection for maternal effects was significantly stronger than selection for direct effects, especially when there was a strong negative correlation between the effects. We see the reason in the fact that the BLUP breeding value estimation worked more accurately for the maternal effect than for the direct effect. For $\sigma_{A,md} = -0.25$, the accuracies of the estimated breeding values in the worker groups were 0.45 for the direct and 0.65 for the maternal effect. In the simulations with $\sigma_{A,md} = -0.75$, the respective values were 0.33 and 0.56. Hence a larger difference in the accuracy of estimated breeding values also yielded a larger difference in selection response. When the genetic variance of the direct effects exceeds one and a half times the genetic variance of the maternal effects ($\sigma^2_{A,d} = 1.8$, $\sigma^2_{A,m} = 1.2$), the selection focus shifted to the direct effects. The reason is that the trait with a higher additive variance has an overproportionally high influence on the outcome of the sum of two traits.

[4] In the published version, this sentence is interchanged with the legend of Figure 3.5. They are now swapped so that the figure can be understood independently.

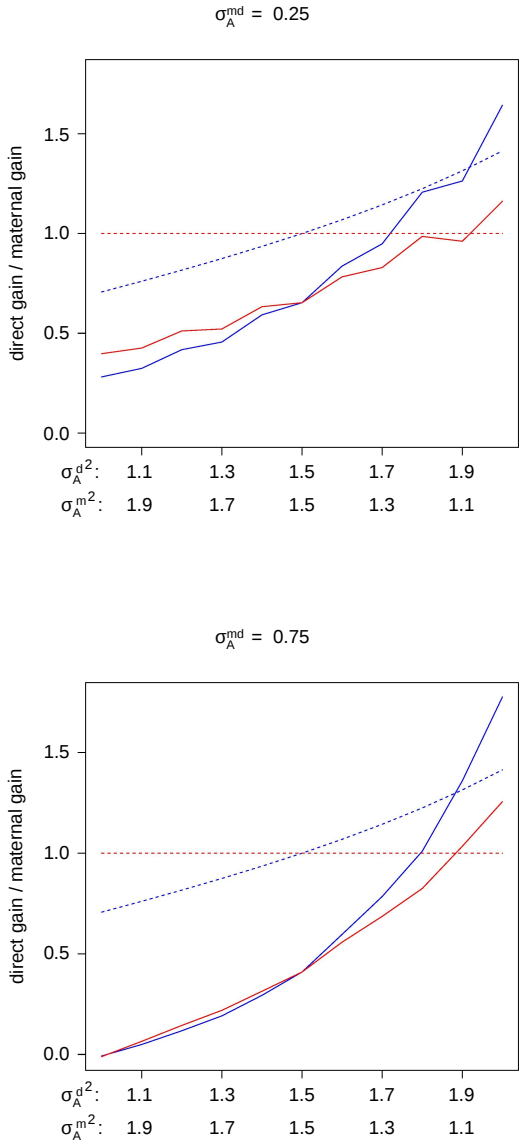

Figure 3.5: **Ratio of direct vs. maternal genetic gain.** Ratio of direct vs. maternal genetic gain after 50 years before (blue) and after (red) correction for the different genetic standard deviations. The dotted lines indicate the ratios of genetic standard deviations.

3.9 References

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Chapter 4

The importance of controlled mating in honeybee breeding

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Abstract

Controlled mating procedures are widely accepted as a key aspect of successful breeding in almost all animal species. In honeybees, however, controlled mating is hard to achieve. Therefore, there have been several attempts to breed honeybees using free-mated queens. In such breeding schemes, selection occurs only on the maternal path since the drone sires are random samples of the population. The success rates of breeding approaches without controlled mating have so far not been investigated on a theoretical or simulation-based level.

Stochastic simulation studies were carried out to examine the chances of success in honeybee breeding with and without controlled mating. We investigated the influence of different sizes of breeding populations (500, 1000, 2000 colonies per year) and unselected passive populations (0, 500, 1000, 2000, infinitely many colonies per year) on selection for a maternally (queen) and directly (worker group) influenced trait with moderate ($r_{md} = -0.53$) or strong ($r_{md} = -0.88$) negative correlation between the two effects. The simulations described 20 years of selection.

Our simulations showed a reduction of breeding success between 47 and 99% if mating was not controlled. In the most drastic cases, practically no genetic gain could be generated without controlled mating. We observed that in the trade-off between selection for direct or maternal effects, the absence of mating control leads to a shift in favor of maternal effects. Moreover, we describe the implications of different breeding strategies on the unselected passive population that benefits only indirectly via the transfer of queens or drones from the breeding population. We show that genetic gain in the passive population develops parallel to that of the breeding population. However, we found a genetic lag that became significantly smaller as more breeding queens served as dams of queens in the passive population.

We conclude that even when unwanted admixture of subspecies can be excluded in natural matings, controlled mating is imperative for successful breeding efforts. This is especially highlighted by the strong positive impact that controlled mating in the breeding population has on the unselected passive population.

4.1 Background

The beginning of the modern era of animal breeding owes everything to Sir Robert Bakewell (1725–1795). Sir Bakewell combined strict record-keeping with the intentional mating of closely related animals for the expression of desirable traits in the population. His breeding strategies proved successful and were soon copied across Europe and North

America [1–3]. An important factor in his success was the strict separation of male and female individuals except for mating, which took place under controlled conditions. Today, controlled mating is still a crucial factor in successful animal breeding. Species for which controlled mating is hard to achieve, such as aquaculture species, lag behind in breeding success partly for this reason [4, 5]. A lack of controlled mating is also a common inhibitory factor in successful animal breeding in developing countries [6, 7]. Modern breeding strategies, involving genetic evaluation, rely heavily on reliable pedigree data, further strengthening the importance of controlled mating [8].

In comparison with other agricultural species, controlled mating in the honeybee appears to be especially hard to achieve. A few days after hatching, a young honeybee queen will undertake one or several nuptial flights to drone congregation areas during which she mates in mid-air with an average of 12 drones from neighboring hives [9]. The exact number and origin of the drones cannot usually be observed [10]. The necessity of mating control became first apparent in the middle of the 19th century when the Italian honeybee (*Apis mellifera ligustica*) was introduced to Switzerland (1843), Germany (1853), and the United States (1860) [11, 12]. Previously, only the dark honeybee (*A. m. mellifera*) had been maintained in these areas [12, 13]. In the middle of the 19th century, controlled mating was not seen as a means of selective breeding but rather a means of avoiding the newly introduced subspecies to cross with the native population [13]. Strategies for controlled mating involved unsuccessful efforts of tethering the queen or enclosing the queen and drones in a tent. Attempts to achieve controlled mating by delaying the flight time of queens and drones to avoid the time window of other drones' natural flight were more successful [11, 14, 15]. This practice, which has recently been rediscovered and became known as the Horner system, was occasionally used in Germany and the United States in the late 19th century [13, 15, 16]. Mating control via geographic isolation of the queen and the desired drones on so-called isolated mating stations was first (unsuccessfully) attempted by T. C. von Baldenstein in Switzerland in 1848 [17]. During the end of the 19th century, this technique was repeatedly applied by Swiss beekeepers, mainly under the leadership of U. Kramer from Zurich [13, 14, 18]. The concept of isolated mating stations has henceforth been developed further and is very popular in Central European honeybee breeding to this day [19]. The first reports of successful artificial inseminations of honeybee queens date back to the late 19th century. During the 1940s and 1950s, artificial insemination was further developed into a practical tool in economic bee breeding and is still used today [13, 14, 20].

Controlled matings allowed beekeepers to keep accurate stud books including pedigree and performance information for use in directional breeding. The systematic collection of such data was introduced in Germany around 1950 [21, 22]. In 1994, the best linear unbiased prediction (BLUP) methodology [8] was adapted to the honeybee [23] and has since yielded significant genetic improvement in all selection traits [24]. In the course of the SmartBees project [25], breeding efforts have begun in numerous European countries after the development of standardized performance testing protocols [26, 27]. To date, infrastructure for controlled mating has not been created in many European regions and its introduction will be connected with considerable logistic efforts. As most local breeders have not had any experience with controlled mating so far, it is unclear how willingly they are going to invest in these extra expenditures. Therefore the following question arises: is successful breeding possible without controlled mating by selecting dam queens only? In this context, it might be beneficial for a breeder to distribute colonies from her/his own stock among the neighboring beekeepers to increase the probability of his/her own queens mating with drones carrying good genetic material.

In this study, we compare the genetic progress in honeybee populations undergoing selection with either free mating or mating on isolated mating stations. The uncontrolled mating procedure makes it necessary to consider an unselected passive population besides the breeding population and the possible exchange of queens and drones between the populations. In addition to the genetic progress in the breeding population, this set-up also allows for further investigations on the individual contribution of maternal and direct effects under various breeding conditions. Furthermore, we examined how breeding and passive population affect each other and in particular, how the passive population can benefit from changes in the breeding population. The situation of two or more partially connected populations following different selection principles has previously been studied in other agricultural species in the context of nucleus breeding schemes both theoretically [28, 29] and by simulations [30, 31]. However, to our knowledge, there has been no such study for the honeybee with its biological peculiarities. Furthermore, none of the studies we are aware of explicitly explored the role of controlled mating in animal breeding schemes.

4.2 Methods

We used the program BeeSim [32] to simulate the construction of honeybee populations consisting of queens and their workers as well as drones. All simulated queens belonged to one of three mutually exclusive categories:

- Breeding queens (BQ) were queens whose colonies underwent performance tests and that were subject to selection.
- Drone producing queens (DPQ) were queens that produced the drones with which BQ could mate on a mating station.
- Passive queens (PQ) formed the unselected passive population. They did not undergo any performance testing, breeding value estimation or selection procedure.

We simulated various population sizes of the breeding population and passive population. We assumed that the numbers of BQ and PQ born in each year were constant and referred to those numbers as N_b and N_p, respectively. We considered the values $N_b = 500$, $N_b = 1000$, and $N_b = 2000$, as well as $N_p = 0$, $N_p = 500$, $N_p = 1000$, $N_p = 2000$, and $N_p = \infty$. In the case of controlled mating, we considered different numbers N_s of mating stations. In particular, we simulated $N_s = 5$, $N_s = 10$, or $N_s = 20$ mating stations per year, each equipped with a sister group of eight DPQ. By stating that $N_s = 0$, we indicate that no controlled mating took place. Mating stations correspond with sires in other species [23, 32–34]; the relatively small values for N_s are realistic due to the large logistic efforts of station maintenance.

All dams of breeding queens were necessarily breeding queens themselves. However, for the passive population we assumed that PQ could have dams from either population and considered different relative proportions q of PQ that had a dam from the breeding population. We simulated the different rates $q = 0$, $q = 0.25$, $q = 0.5$, $q = 0.75$, and $q = 1$.

We selected for a directly (worker group) and maternally (queen) affected quantitative trait with an additive maternal genetic variance of $\sigma^2_{A,m} = 1$, an additive direct variance of $\sigma^2_{A,d} = 2$, and a residual variance of $\sigma^2_E = 1$. We chose two different values for the correlation between the effects: one set of simulations was run with a medium negative correlation of $r_{md} = -0.53$ (i. e. covariance $\sigma_{A,md} = -0.75$) and another set with a stronger negative correlation of $r_{md} = -0.88$ ($\sigma_{A,md} = -1.25$). These numbers correspond to maternal and direct heritabilities of $h^2_m = 0.53$, $h^2_d = 0.37$ in the case of $r_{md} = -0.53$,

and $h_m^2 = 0.72$, $h_d^2 = 0.46$ in the case of $r_{md} = -0.88$ (see [35] for a detailed description of the calculation of heritabilities for honeybees, where the direct effect reflects the mean of the worker group.) The chosen numbers roughly represent those reported in the literature for parameter estimates for economically important traits such as honey yield or swarming behavior [35, 36].

All possible combinations of the parameters N_b, N_p, N_s, q, and r_{md} were simulated separately over the course of 20 years and repeated 100 times in order to obtain stable results. An exception formed the parameter choices $N_p = 0$ and $N_p = \infty$, which were only simulated in combination with $q = 0$, leading to a total number of 408 simulation settings (see Table 4.1).

N_b	N_p	N_s	q	r_{md}
500	0*	0**	0	-0.53
1000	500	5	0.25	-0.88
2000	1000	10	0.5	
	2000	20	0.75	
	∞*		1	

* only in combination with $q = 0$.
** indicates uncontrolled mating.

Table 4.1: **Overview of the parameters used for the simulations.** N_b: number of breeding queens per year. N_p: number of passive queens per year. N_s: number of isolated mating stations. q: relative proportion of rest queens with breeding queen dams. r_{md}: correlation between maternal and direct effect.

The animals' genetics were simulated for a directly and maternally influenced trait according to an infinitesimal model that accounts for the haploid nature of drones. Queens of the base population were equipped with direct and maternal true breeding values following a normal distribution with mean 0 and variance $\mathbf{\Sigma}_A$ given by $\sigma^2_{A,m}$, $\sigma^2_{A,d}$, and $\sigma_{A,md}$. The inheritance of true breeding values from a queen Q to a drone D was realized as:

$$\mathbf{TBV}_D = \mathbf{TBV}_Q + \sqrt{1 - F_Q} \cdot \mathbf{\Phi}, \tag{4.1}$$

and from a queen Q and drone D to an offspring queen R as:

$$\mathbf{TBV}_R = \frac{1}{2}\left(\mathbf{TBV}_D + \mathbf{TBV}_Q\sqrt{1 - F_Q} \cdot \mathbf{\Phi}\right). \tag{4.2}$$

Hereby, F_Q denotes the inbreeding coefficient of Q and $\mathbf{\Phi}$ denotes a $N(\mathbf{0}, \mathbf{\Sigma}_A)$-distributed Mendelian sample term. Finally, worker groups obtained their breeding values as the mean value of their queen's TBV and the average TBV of their sire drones. See [32] for a detailed description of the infinitesimal model for honeybees.

4.2.1 Breeding population

When mating took place in a controlled manner, the breeding population was simulated as described in [32], i.e., each breeding queen produced a worker group and underwent performance testing. Furthermore, each year the best 20% of two-year-old breeding queens based on a BLUP evaluation were selected as dams and each produced five breeding queens as offspring. The N_s best three-year-old queens each produced a sister group as set-up for a mating station on which the newly created queens were mated with 12 drones each. In the case of uncontrolled matings, breeding queens mated with 12 drones whose dams, one to three years old, were picked randomly from the entire (i.e. breeding and passive) population. Contrary to usual beekeeping practice, we did not simulate any culling or other exclusions of queens, so that one, two and three year old queens were equally represented among the dams of the drones. Thus, the probability of a drone in such a mating to come from a breeding colony was $p = \frac{N_b}{N_b+N_p}$. In the case of an infinite passive population, the dams of the drones were selected exclusively from the passive population. Also when matings were uncontrolled, a BLUP evaluation was performed each year and the best 20% of breeding queens were selected to produce five daughters each.
Regardless of the mating procedure, the true breeding values were passed according to Eqs. 1 and 2. However, when the inverse additive relationship matrix for the BLUP evaluations was calculated, only the information that is available in reality was taken into account. In particular, with uncontrolled mating, all sires were assumed to be unknown. The inverse additive relationship matrix was calculated with the bee specific approach of [37] following the ideas of [33], which combines the classical inversion strategy of Henderson [38] with the bee specific properties of haploid drones while considering the complex situation on mating stations.

4.2.2 Passive population

After being generated, each queen of the passive population mated with 12 drones whose dams, one to three years old, were picked randomly from the entire (i.e. breeding and

passive) population. Thus, the probability that a drone in such a mating comes from a breeding colony was also $p = \frac{N_b}{N_b+N_p}$, as in a breeding population without controlled mating.

When it came to generating new passive queens, a relative proportion q of these was created as offspring of randomly chosen BQ one to three years old. For the remaining passive queens, a dam was randomly selected among the passive queens one to three years old. The maternal generation interval for the passive queens was thus chosen to be more variable than that of the breeding population since these colonies do not have to follow the somewhat strict schedule of performance testing and subsequent selection.

The passive population was not included in the BLUP breeding value estimation and no worker groups were simulated for the passive queens. However, queens and drones from the passive population did inherit and pass on true breeding values.

Infinite passive population

Unlike the finite passive populations, the infinite passive population was not explicitly simulated. We assumed that, in an infinite passive population which does not receive any queens from the breeding population ($q = 0$), the passive population would not undergo any genetic changes due to selection efforts in the breeding population. Thus, the passive population was only needed when breeding queens were left to mate without control. For that purpose, drones were created as if they belonged to the base population.

4.3 Results

4.3.1 Genetic progress in the breeding population

We investigated how the queens' true breeding values changed over the course of 20 years. In all simulated settings, the average true breeding value in the first two years was close to zero, since the breeding queens of these years formed the base population. In years three to five, a genetic response was observed. We defined the total breeding value of a queen as the sum of her direct and maternal breeding values. In the models with a moderately negative correlation between direct and maternal effects, $r_{md} = -0.53$, the accumulated gain in total breeding values of queens in year five was between 0.63 and 0.81 units with little differences between the population sizes and breeding schemes. With a strong negative correlation, $r_{md} = -0.88$, a genetic response of 0.12 to 0.23 units could

be accumulated by year five. In both cases, settings with controlled mating performed slightly better. After the first five years, genetic gain increased nearly linearly in all settings. However, the rate of genetic improvement varied drastically among the different set-ups. On average, without controlled mating, the genetic gain from year 5 to year 20, measured in total breeding values of queens, was 75% lower than with controlled mating. Based on the individual setting, the range of reduction in breeding success was from 47% to 99% (see Figure 4.1).

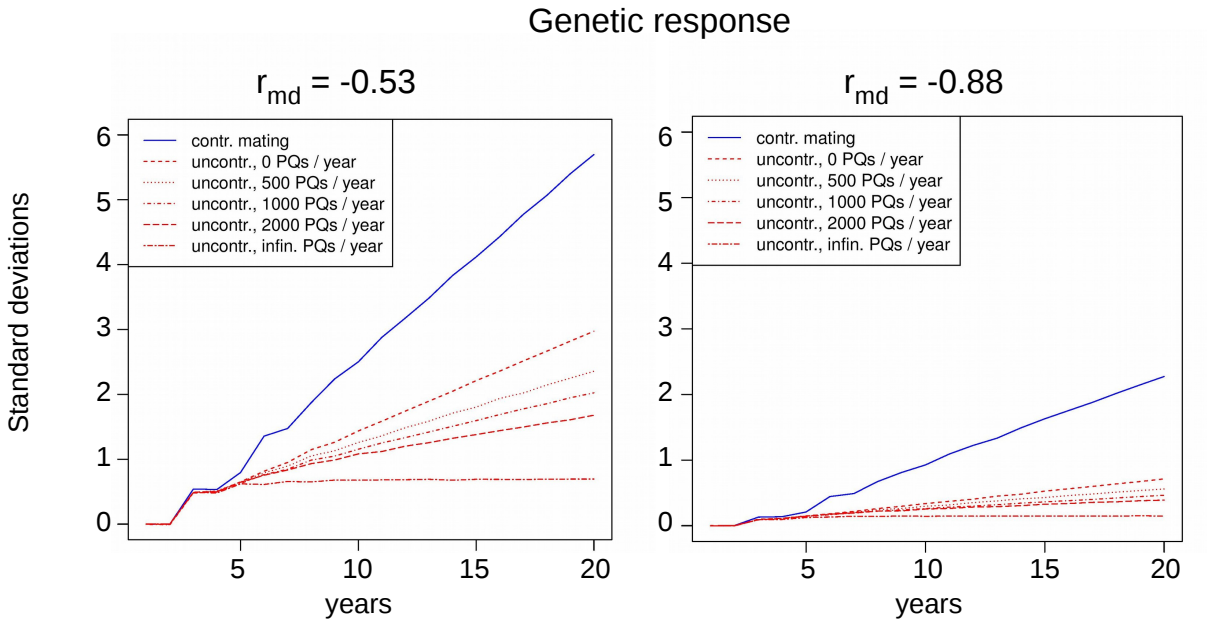

Figure 4.1: **Genetic response with and without controlled mating.** Genetic response to selection over the course of 20 years with a moderate ($r_{md} = -0.53$) and strong ($r_{md} = -0.88$) negative correlation between maternal and direct effects. Results are shown for a breeding population of 1000 colonies per year and various passive population sizes with (solid blue line) and without (dotted red lines) controlled mating. The passive population did not receive any dams from the breeding population ($q = 0$).

4.3.2 Impact of population proportions

While all breeding schemes with uncontrolled mating performed significantly worse than their counterparts with controlled mating, the difference was more marked, when the ratio between BQ and PQ was small (see Figure 4.1, Table 4.2). The data suggest, that, without controlled mating, breeding success depends linearly on the relative proportion

p of BQ in the total population, i.e. $\frac{N_b}{N_b+N_p}$. The correlation between this value and the genetic gain between years 5 and 20 was 0.993 for $r_{md} = -0.53$ and 0.996 for $r_{md} = -0.88$.

		contr. mating	uncontrolled mating				
			0 PQ	500 PQ	1000 PQ	2000 PQ	∞ PQ
$r_{md} = -0.53$	500 BQ	4.68	2.31	1.37	1.07	0.74	0.07
	1000 BQ	4.92	2.32	1.71	1.40	1.03	0.07
	2000 BQ	5.08	2.34	1.98	1.72	1.40	0.08
$r_{md} = -0.88$	500 BQ	1.79	0.56	0.32	0.24	0.19	0.02
	1000 BQ	2.04	0.56	0.41	0.32	0.25	0.02
	2000 BQ	2.19	0.56	0.47	0.40	0.33	0.02

Table 4.2: **Genetic gain under controlled vs. uncontrolled mating.** Genetic gain from year 5 to 20 in settings with controlled and uncontrolled mating for different correlations between direct and maternal effects (r_{md}) and various sizes of breeding and passive populations. Numbers for controlled mating are averages over simulation outcomes for all positive values of N_s.

When parts of the passive queen population had dams from the breeding population ($q > 0$), the genetic response without controlled mating could be slightly improved. The improvements in genetic gain due to positive values of q were stronger when the breeding population was small compared to the passive population: In the setting with 500 BQ and 2000 PQ the breeding success was roughly doubled from $q = 0$ to $q = 1$, whereas in the setting with 2000 BQ and 500 PQ, there was only an improvement of between 7 and 8%. In no case, however, could the improvement of genetic gain due to $q > 0$ make up for the negative effects of the lack of controlled mating (see Table 4.3).
When mating took place in a controlled manner, the realized number of mating stations had only a minor effect on the genetic gain after 20 years (see Table 4.4). Different numbers of mating stations caused deviations of up to 4.8% in the simulations with a moderately negative correlation between direct and maternal effects. In the simulations with a strong negative correlation, the deviations ranged up to 10.5%. There was no clear indication for an ideal number of mating stations. Traits with a stronger negative correlation ($r_{md} = -0.88$) tended to prefer more mating stations than traits with moderate negative correlation ($r_{md} = -0.53$).

		$r_{md} = -0.53$						$r_{md} = -0.88$					
		uncontrolled, $q =$					contr.	uncontrolled, $q =$					contr.
BQ	PQ	0.0	0.25	0.5	0.75	1.0		0.0	0.25	0.5	0.75	1.0	
	500	1.37	1.54	1.66	1.72	1.77	4.67	0.32	0.36	0.39	0.39	0.43	1.77
500	1000	1.07	1.23	1.38	1.51	1.58	4.71	0.24	0.29	0.33	0.35	0.37	1.78
	2000	0.74	1.02	1.17	1.34	1.42	4.71	0.19	0.25	0.28	0.31	0.34	1.80
	500	1.71	1.82	1.88	1.93	1.97	4.93	0.41	0.43	0.44	0.46	0.47	2.02
1000	1000	1.40	1.54	1.65	1.73	1.78	4.92	0.32	0.36	0.38	0.41	0.42	2.03
	2000	1.03	1.24	1.38	1.51	1.60	4.96	0.25	0.29	0.33	0.36	0.38	2.02
	500	1.98	2.04	2.07	2.09	2.13	5.07	0.47	0.48	0.49	0.50	0.50	2.19
2000	1000	1.72	1.81	1.89	1.93	1.98	5.07	0.40	0.43	0.44	0.45	0.46	2.20
	2000	1.40	1.55	1.64	1.73	1.79	5.08	0.33	0.36	0.38	0.41	0.42	2.19

Table 4.3: **Influence of parameter q.** Genetic gain from year 5 to 20 in in the breeding population without controlled mating when different proportions q of the PQ have dams from the breeding population. The corresponding rates of gain with controlled mating are given for comparison.

	$r_{md} = -0.53$			$r_{md} = -0.88$		
BQ per year	$N_s = 5$	$N_s = 10$	$N_s = 20$	$N_s = 5$	$N_s = 10$	$N_s = 20$
500	4.70	4.61	4.40	1.78	1.90	1.89
1000	4.96	4.93	4.76	1.86	2.02	2.05
2000	5.21	5.20	5.07	1.94	2.12	2.19

Table 4.4: **Influence of number of mating stations.** Genetic gain from year 5 to 20 in different settings with controlled mating.

4.3.3 Direct and maternal effects

Different simulation settings not only led to different genetic gains in total breeding values, but also had great impact on the subdivision of total genetic gain into direct and maternal genetic gain (see Figure 4.2). In the following two paragraphs, we describe the progress for controlled and uncontrolled matings, separately.

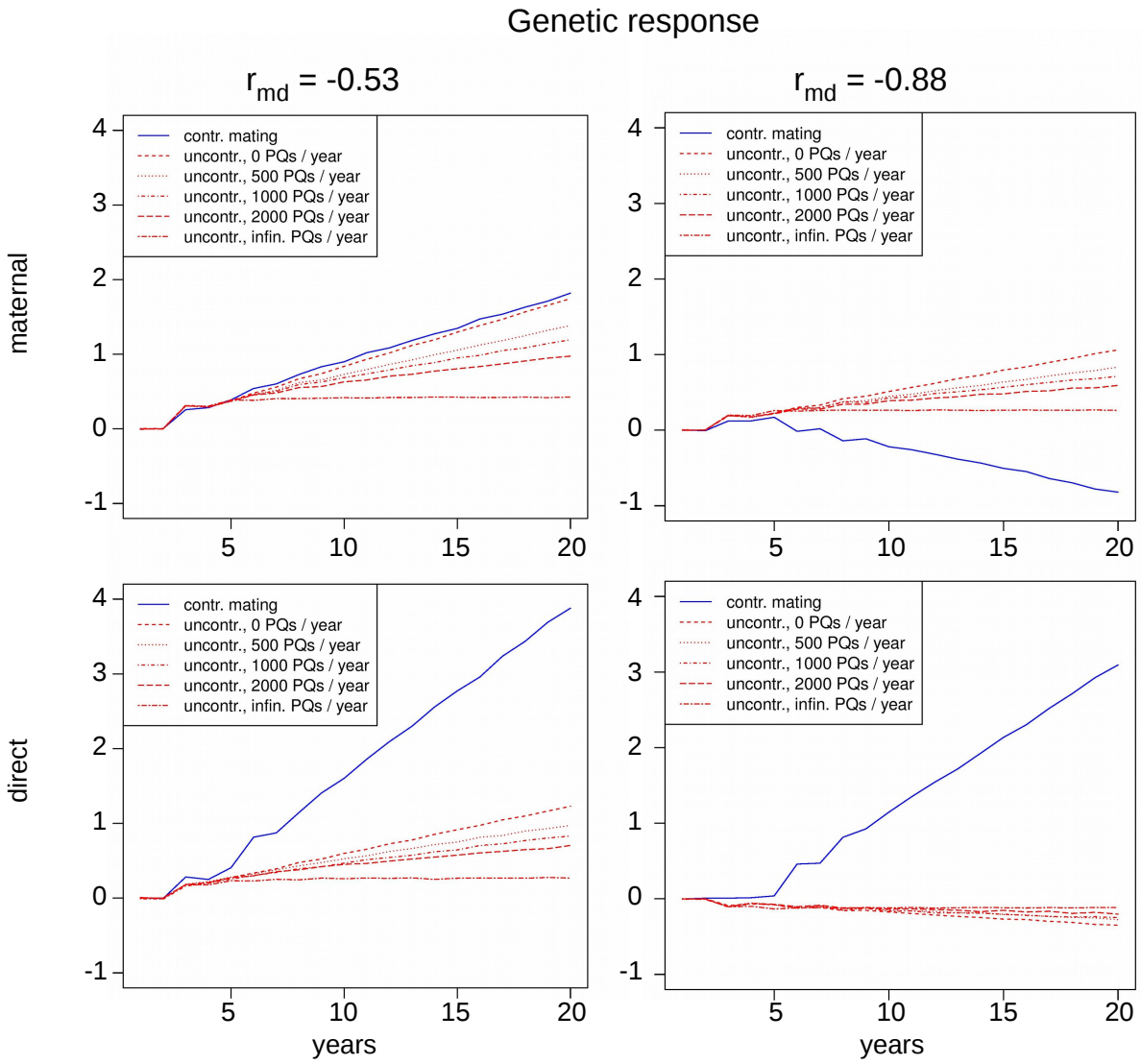

Figure 4.2: **Genetic response for direct and maternal effects.** Genetic response to selection of maternal and direct effects over the course of 20 years with a moderate ($r_{md} = -0.53$) and strong ($r_{md} = -0.88$) negative correlation between maternal and direct effects. Results are shown for a breeding population of 1000 colonies per year and various passive population sizes with (solid blue line) and without (dotted red lines) controlled mating. The passive population did not receive any dams from the breeding population ($q = 0$).

Controlled mating

In simulations with controlled mating, the genetic gain for the direct effects was greater than for the maternal effects. When the correlation between direct and maternal effects

was moderate ($r_{md} = -0.53$), the ratio between direct and maternal genetic gain from years 5 to 20 was between 2.01 and 2.88. Larger numbers of mating stations led to a stronger selection focus on direct effects.
A strong negative correlation ($r_{md} = -0.88$) even led to negative selection on maternal effects. However, the negative change in the maternal effects was outweighed by positive selection on direct effects by a factor between 2.77 and 5.07. Here, the selection on direct effects was stronger, when there were fewer mating stations.

Uncontrolled mating

With uncontrolled mating, the selection focus switched to the maternal effects. With a moderate negative correlation, the ratio between maternal and direct gain was between 1.29 and 1.60 without clear dependencies on population sizes or relative proportion of passive queens with breeding queen dams (q).
A strong negative correlation ($r_{md} = -0.88$) yielded a slightly negative selection on direct effects, which was outweighed by the gain in the maternal effects by a factor between 2.46 and 3.39. Again, no clear dependencies could be detected.

4.3.4 Changes in the passive population

All finite passive populations showed a positive genetic response which was delayed in time compared to the breeding population. When breeding queens served as dams for at least half of the passive queens ($q \geq 0.5$), the genetic difference between breeding and passive population remained constant after a few years (see Figure 4.3). For $q = 1$ and controlled mating conditions in the breeding population, the passive population stayed between 1.47 and 2.03 years behind the breeding population in terms of genetic gain. The time delay was shorter for smaller breeding populations. When q was reduced to 0.5, it took the passive population between 3.17 and 4.24 years to reach the level of the breeding population. Here, smaller passive populations led to a smaller gap between the breeding values of the respective populations. Under uncontrolled mating conditions for the breeding population, the time difference in genetic gain increased to between 3.26 and 5.16 years for $q = 1$, respectively to between 4.56 and 8.67 years for $q = 0.5$. For values of $q < 0.5$ and in particular for $q = 0$, i.e. no breeding queen dams for the passive population, the genetic gain of the passive population was slower than that of the breeding population throughout the entire 20 years. However, in these cases the genetic response in

the passive population was superlinear over the entire 20 years. Thus, it is expected that the passive population will also reach the rates of genetic gain of the breeding population, but only well after 20 years.

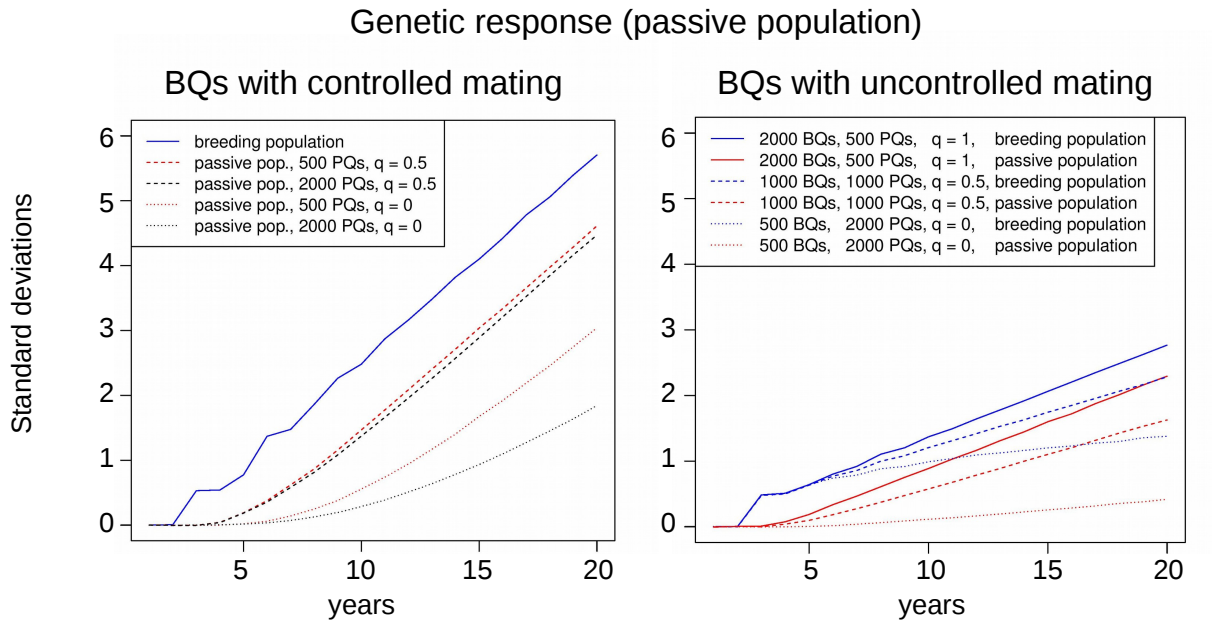

Figure 4.3: **Genetic response in the passive population.** Genetic response to selection in the breeding and passive populations over the course of 20 years with a moderate ($r_{md} = -0.53$) negative correlation between maternal and direct effects. Results are shown for a breeding population with (left hand side) or without (right hand side) controlled mating, different population sizes and different relative proportions of passive queens with breeding queen dams (q).

4.4 Discussion

4.4.1 Model choice

Genetic model and simulated time

Simulation studies in animal breeding mostly rely on either Fisher's infinitesimal model [39, 40] or on finite locus models [41]. Previously, we have shown in the context of honeybee breeding that long-term simulation studies based on finite locus models are more reliable than those that use the infinitesimal model [32]. However, in the same study, we also showed that for studies that do not exceed the timeframe of 20 years, either model works

equally well. Thus, we decided to use the infinitesimal model because it has fewer levels of freedom and therefore needs fewer repetitions of the simulations to obtain stable results. The simulated time of 20 years, i. e. 10 maternal generations, is rather short for an investigation of strategies in animal breeding [42, 43]. However, such limited time frames are not without examples [44, 45] and may even be long considering the objectives of individual breeders.

In the long term, breeding schemes with uncontrolled mating will generate lower rates of inbreeding and thus a reduced loss of genetic variance. However, promising mating schemes in animal breeding that aim at avoiding high inbreeding rates, such as optimum contribution selection, generally do not compromise genetic gain to a large extent [43, 46]. Based on our current findings, it is already clear that this is not the case for uncontrolled mating of honeybees.

In order to hint at the long-term effects of controlled and uncontrolled mating, we conducted a small-scale simulation with 20 repetitions over the course of 100 years. (see Figure 4) In this simulation, we chose the parameters $N_b = 500$, $N_p = 1000$, $r_{md} = -0.53$, and $q = 0.5$ and uncontrolled mating ($N_s = 0$) or controlled mating on $N_s = 20$ mating stations. As a genetic model, we chose a finite locus model with 400 unlinked loci as is described in [32]. After 100 years, the genetic response in the simulation without controlled mating was reduced by 43% in comparison to the controlled mating scheme. Without controlled mating, the initial standard deviation was reduced by only 24% (63% with controlled mating). In another simulation, we reduced the loss of genetic standard deviation under controlled mating by increasing the number of mating stations to 50 and selecting 50% of all breeding queens as dams (as opposed to 20% in the rest of our simulations). By this means, we could decrease the loss of genetic standard deviation from 63 to 43% while the genetic response after 100 years decreased by only 5%. We expect that an implementation of more sophisticated selection srategies, such as optimum contribution selection [46], can yield high response rates with even smaller reductions of variance. Thus, in our opinion renouncing controlled mating is a clearly inferior breeding practice for honeybees, also in the long term.

Natural selection

In the honeybee, resistance to the parasitic *Varroa* mite is a trait that is often regarded to be a fitness advantage [47, 48] and some breeding strategies rely on the assumption that drones from resistant colonies are more likely to reproduce than those from susceptible

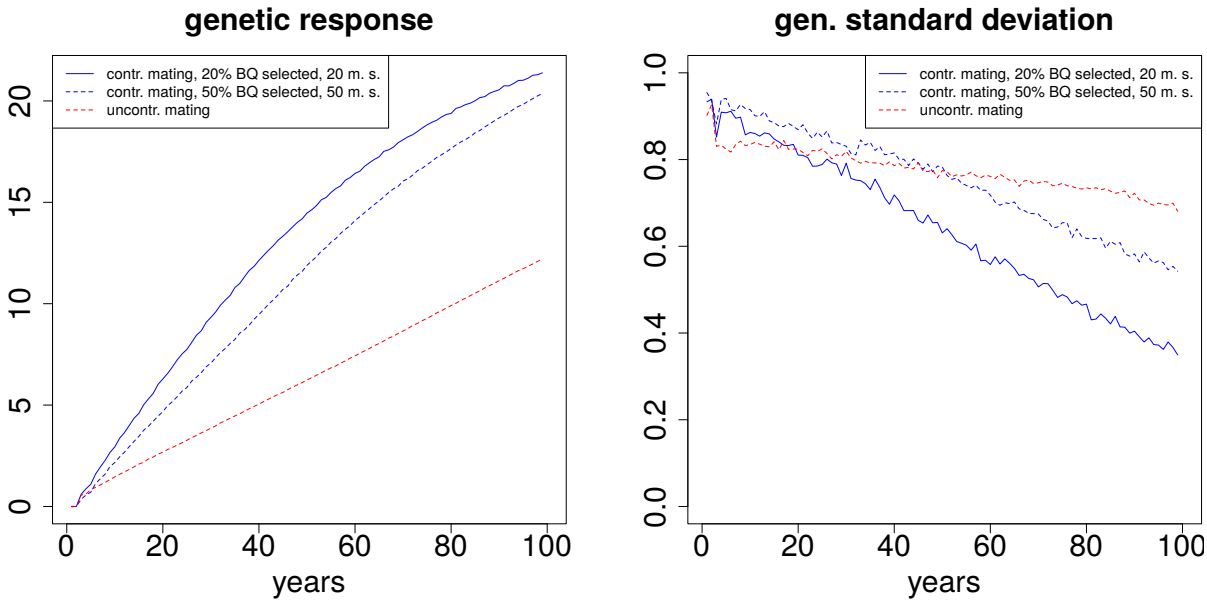

Figure 4.4: **Genetic change over 100 years.** Genetic response to selection (left hand side) and evolution of genetic standard deviation (right hand side) over the course of 100 years with a moderate ($r_{md} = -0.53$) negative correlation between maternal and direct effects. Results are shown for a breeding population of 500 colonies and a passive population of 1000 colonies per year with (blue lines) and without (dotted red line) controlled mating. Controlled mating was performed with two different selection intensities: 20 mating stations and the best 20% of BQ selected as dams (solid blue line) of 50 mating stations and the best 50% of BQ selected as dams. We assumed $q = 0.5$.

colonies [19, 49]. Other commercial quantitative traits in the honeybee, such as honey production or gentleness, appear to have negligible implications on colony fitness.
In our studies, we assumed an absence of natural selection against or in favor of the simulated trait and thus that our simulations accurately describe the situation for most commercial traits. For the *Varroa* resistance trait, the absence of controlled mating may be less inferior than our simulations suggest since in uncontrolled matings resistant drones also have a fitness advantage over susceptible drones. In small populations, the so-called tolerance mating stations as they are described in [49, 50] may be a promising alternative to classical mating stations for this trait.

Age structure

In our simulations, all queens between one and three years old had the same chance of being the dam of a drone involved in an uncontrolled mating, and the number of queens

was the same in each age group. Similarly, the dams of passive queens were chosen randomly from queens one to three years old. In reality, there will be more younger queens because several queens will not reach three years of age due to illnesses, colony losses or requeening practice [26, 51]. Furthermore, it is likely that in reality, the queen-to-queen generation interval in the passive population will be larger than in the breeding population because in the passive population there is no urge to rapidly improve the genetic material. This effect will become even stronger in the future due to the possibilities of shorter generation intervals due to genomic selection [34, 52]. However, this study aimed at investigating the implications of controlled mating on breeding success. In order to be able to quantify its effects, we avoided intermingling them with other factors such as age structures or generation intervals. The fact that queens of all ages are equally represented leads to an average queen-to-queen generation interval in the passive population of two years, which equals the queen-to-queen interval in the breeding population. This facilitates the comparison between the breeding and passive populations.
Simulation studies with realistic age structures and generation intervals would require a detailed knowledge of the behavior of beekeepers of the passive population. However, due to the nature of the passive population, no such data is available. In areas where beekeeping is mainly carried out by large commercial operatiors, procedures are likely to be highly standardized. However, the diverse structure of beekeeping in Europe with many recreational beekeepers suggests a wide variety in individual practices. Nevertheless, we expect that realistic simulations would lead to results that are similar to those presented here.

Total breeding value

In accordance with most of the literature on bee breeding, the sum of the direct and maternal breeding values of a worker group served as the selection criterion in the present simulations. This value is generally chosen since it represents the expected genetic properties of an unmated queen offspring of the colony [23, 33, 53].
However after simulation, when we investigated the change in the genetic response in the population, we chose the sum of the direct and maternal breeding values of a queen as the total breeding value. This choice has the advantage that it allows comparing genetic progress between breeding and passive populations even though PQ are generally simulated without worker groups. Based on our previous work, [32], the selection criterion

and the total breeding value as defined here generally show the same behavior. The present definition of total breeding values has previously been used in [54].

4.4.2 Impact of controlled mating

The linear genetic progress is in line with other breeding simulations in the literature [32, 55]. Furthermore, the rate of genetic gain in a trait with a moderate negative correlation between direct and maternal effects ($r_{md} = -0.53$) under controlled mating conditions is similar to the results in [32].
To our knowledge, these are the first simulation studies on breeding strategies without mating control. We found that the absence of controlled mating clearly impaired genetic progress in all settings in two ways.

- First, it allows only for inaccurate calculations of relationships and therefore leads to a less reliable BLUP-based breeding value estimation [33].
- Second, it does not allow for selection on the paternal path and the genetic progress will continuously be hampered by queens that mate with genetically inferior drones from the breeding or passive population.

Since the drones in the passive population can not or only indirectly benefit from the breeding efforts, the risk of queens mating with undesired genetic material increases with the relative proportion of PQ in the population. The high correlation rates between the relative proportion of BQ in the population and the rate of genetic gain indicate that the impact of genetically inferior drones is the prevalent factor.
If breeders give away virgin queens to the passive population ($q > 0$), this improves the average genetics of the passive population. However, this affects the breeding population only whenever a BQ mates with a drone from the passive population. This explains, why the positive influence of a positive q value was especially high, when the passive population was relatively large. The improvements in the breeding population due to $q > 0$ were generally small, which has its reason in the indirect nature of the effect.
The situation of $q > 0$ can be compared to nucleus breeding programs in other agricultural species, where nucleus-born individuals are disseminated to the base population. In a related setting (albeit with controlled matings with sires of the base population), James [28] also derived a small positive influence of such practices.

An extreme case: infinite passive population

In the case of uncontrolled mating with an infinite passive population that did not receive queens from the breeding population ($q = 0$), there was little extra genetic gain after a few years (see Figure 4.1, Table 4.2). This may seem surprising at first sight as one might think that the selection of superior dam queens would have to lead to at least some improvement. However, there is a theoretical explanation to this effect as described below.

Let $\overline{\mathrm{TBV}}_t$ be the average breeding value of the breeding population in year t. Then, since we assumed an absence of selection in the passive population and that it cannot benefit from the breeding population ($q = 0$, $p = \frac{N_b}{N_b+\infty} = 0$), the average breeding value of the infinite passive population remains constant $\overline{\mathrm{TBV}}_0$. Furthermore, we assumed that the average breeding value of the **selected** breeding queens in year t is $\overline{\mathrm{TBV}}_t + S_t$ and that $S_t \leq S$ is bounded for all years. Now, once the average breeding value of the breeding population has improved by this upper bound S,

$$\overline{\mathrm{TBV}}_{t_0} = \overline{\mathrm{TBV}}_0 + S,$$

we obtain, for the next generation, the average of the selected queens of year t_0 and the drones from the passive population. I.e.,

$$\begin{aligned}
\overline{\mathrm{TBV}}_{t_0+\text{generation interval}} &= \frac{1}{2}\left(\overline{\mathrm{TBV}}_{t_0} + S_{t_0} + \overline{\mathrm{TBV}}_0\right) \\
&\leq \frac{1}{2}\left(\overline{\mathrm{TBV}}_0 + S + S + \overline{\mathrm{TBV}}_0\right) \\
&= \overline{\mathrm{TBV}}_0 + S
\end{aligned}$$

Thus, the average breeding value of the breeding population will never exceed the maximum superiority S of selected dam queens.

Breeding with uncontrolled mating in reality

Several honeybee breeding experiments without controlled mating have been performed to improve the hygienic behavior of workers but the results of these studies are ambiguous. While [56] found only small improvements over five generations, other studies have shown short-term breeding success without controlled mating in the selection for hygienic behavior [57–59]. but did not investigate if the initial rate of genetic improvement after

one generation could be maintained over longer periods of time. In fact, the results of [57] show an initial improvement in the first two years but stagnation afterwards. Like [59], our simulation studies indicated an initial breeding success under uncrontrolled conditions that was only slightly inferior to selection with controlled mating. However, our simulations show that this initial genetic improvement in the first few years cannot be held up in the middle and long term.

Further aspects of controlled mating

In this study, we did not assume that genetic transfer between the breeding and passive populations has any implications beyond the influence on an unspecified trait. This would be the case if breeding and passive population are genetically similar, i.e., belong to the same subspecies. In practice, however, many of the newly established bee breeding programs in Europe are confronted with the situation that the native population is heavily endangered by admixture due to the introduction of foreign honeybee subspecies [60, 61]. Besides the moral aspect of conserving native subspecies, there are also economical reasons to prevent admixture, as it has been observed that hybrids show increased aggressive behavior and native subspecies generally have fitness advantages due to local adaptation [62]. In areas, where there is a risk of crossing between subspecies, controlled mating is crucial beyond reasons of breeding progress [63, 64].
In regions that do not provide the necessary geographical features for secure mating stations, artificial insemination can be a practicable alternative [65]. Furthermore, alternative strategies for controlled mating, using time shifts in the nuptial flights, have shown promising results [16, 66].

4.4.3 Direct and maternal effects

The preferred selection for direct effects under controlled mating conditions is in line with the results of [32] and can be explained by the larger direct additive genetic variance. Negative selection on maternal effects when they are strongly negatively correlated with the direct effects has been shown in simulation studies for other agricultural species [67, 68]. The role of the number of mating stations, which corresponds to the number of sires in other species, can be explained as follows. On the one hand, a small number of mating stations implies a strong selection on the paternal side, which will influence the direct breeding values of the tested worker groups positively. On the other hand, a

larger number of mating stations leads to higher genetic diversity in the sires and thus in the direct effects of the worker groups. This increases the accuracy of the estimation of direct breeding values [69]. Traits with a low negative correlation between direct and maternal effects generally have higher total heritabilities and therefore more accurate breeding values and can thus benefit from an intense selection scheme. In comparison, traits for which direct and maternal effects are strongly negatively correlated need a larger number of sires for an accurate estimation of breeding values.

While maternal effects are expressed directly in the BQ that are to be selected, their direct breeding values can only be deduced via relationship information to their worker groups. This relationship information is far less accurate when uncontrolled mating is applied, which explains the stronger focus on maternal effects. This means, that a part of the reduced genetic gain in selection schemes without controlled mating is also caused by the fact that the direct breeding values cannot be assessed accurately. Therefore, the selection focus is shifted from the ideal mixture of direct and maternal effects and concentrates too strongly on the maternal effects.

The genetic progress in the breeding schemes with controlled mating indicate that with a strong negative correlation between direct and maternal effects it may be ideal to sacrifice the maternal effects in order to overcompensate the maternal genetic loss with gains in the direct effect. When the negative correlation is lower, a positive selection on both effects appears favorable. Future research on how an ideal weighting of the loci under selection on direct and maternal effects depends on their (co-)variances is of great interest.

A negative selection on maternal effects may lead to practical difficulties, even when it is overcompensated by direct genetic gain. It makes the queen more dependent on her own workers which may lead to problems in the practice of queen replacement [70, 71]. However, in practical breeding programs, no negative change of either effect has been observed so far [24, 72].

The genetic parameters in our simulations may appear somewhat extreme. In particular, a negative correlation between effects of $r_{md} = -0.88$ may be seen as too strong and heritabilities of the considered traits are high. We decided to use these parameters because they reflect the estimates that were obtained for economically relevant traits such as honey production or swarming behavior [21, 35]. Negative genetic correlations between direct and maternal effects have repeatedly been estimated for other farm animals and, in some cases, reached or exceeded values around -0.9 [73, 74]. Estimation of parameters for honeybees is particularly difficult because each queen has only one worker group as

offspring that can be used to separate direct effects from maternal effects. In a study on sheep, Maniatis and Pollott showed, that unreasonably strong negative correlations between effects can be estimated when the number of offspring per dam is small and performance data are missing [75]. However, honeybee simulation studies have shown that genetic parameters for honeybees can be estimated without bias [33].
In [32], honeybee breeding simulations with a weaker genetic correlation of $r_{md} = -0.18$ were implemented and showed only quantitative rather than qualitative differences to those with $r_{md} = -0.53$. Thus, we believe that the key results of the present work also hold true if the real correlation between direct and maternal effects is lower than we assumed. In addition, it is most likely that although the breeding values will be estimated with the wrong parameters it will not have a big impact [76].
Traits with indirect genetic effects often show particularly high heritabilities which are caused by the negative correlation between effects. In extreme cases, the genetic variance for individual effects may exceed the phenotypic variance, leading to heritabilities higher than 1, which are impossible in classical theory without indirect effects [77]. In honeybees, this effect is further strengthened by the fact that the direct effect is shown in a collective rather than a single individual, which causes further reduction of the phenotypic variance [35]. Therefore, it is recommended to be cautious when deriving implications from high heritabilities in honeybee traits. For example, Brascamp et *al.* [53] have reported that despite high heritabilities, the selection differentials in honeybee breeding schemes can be seen as low.

4.4.4 Genetic progress in the passive population

To our knowledge, these are the first simulations that investigate the influence of breeding programs on the surrounding unselected population in any agricultural species. However, nucleus breeding programs with interdependent populations have been studied. In these breeding programs, an eventually parallel genetic contribution in the populations has been predicted theoretically [28] and observed in simulations [31]. Therefore, the parallel genetic progress of breeding and passive populations that was observed for $q > 0$ can also be assumed in the case of a maternally self-sufficient passive population when a timeframe of more than 20 years is taken into account. It points out that decisions for the breeding population may have severe consequences for the entire population since they inevitably influence the genetic changes in the passive population with a delay in time. It clearly marks the importance of breeders who use controlled mating, since they will pave the

way for the genetic improvement of the entire population. The results indicate that it is advantageous for beekeepers without breeding ambitions to obtain their queens from active breeders because it lets them benefit from the breeding activities with a shorter time delay.

4.5 Conclusion

Our simulation study shows that controlled mating is crucial to generate genetic response over several generations. Especially in regions where breeders form a minority among the beekeepers, as it is mostly the case, controlled mating is absolutely mandatory. Moreover, depending on the exchange rates of queens and drones, the passive population can also benefit greatly from a controlled mating of BQ. Thus, applying controlled mating does not only mean a personal advantage for individual breeders but is also important for the genetic progress of the passive population.

4.6 References

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Chapter 5

Sustainable breeding of endangered subspecies

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Abstract

Modern breeding structures are emerging for European honeybee populations. However, while genetic evaluations of honeybees are becoming increasingly well understood, little is known about how selection decisions shape the populations' genetic structures. We performed simulations evaluating 100 different selection schemes, defined by selection rates for dams and sires, in populations of 200, 500, or 1000 colonies per year and considering four different quantitative traits, reflecting different genetic parameters and numbers of influential loci. Focusing on sustainability, we evaluated genetic progress over 100 years and related it to inbreeding developments. While all populations allowed for sustainable breeding with generational inbreeding rates below 1% per generation, optimal selection rates differed and sustainable selection was harder to achieve in smaller populations and for stronger negative correlations of maternal and direct effects in the selection trait. In small populations, a third or a fourth of all candidate queens should be selected as dams, whereas this number declined to a sixth for larger population sizes. Furthermore, our simulations indicated that, particularly in small populations, as many sires as possible should be provided. We conclude that carefully applied breeding provides good prospects for currently endangered honeybee subspecies, since sustainable genetic progress improves their attractiveness to beekeepers.

5.1 Introduction

Recent years have seen a notable increase in efforts to improve breeding strategies for the western honeybee (*Apis mellifera*) both theoretically [1–3] and practically [4–7]. As a consequence, for many of the European subspecies, organized breeding activities are being developed for the first time [8–10]. The newly established breeding populations vary greatly in size, ranging from populations with several thousand potential breeding queens per year, which is the case for the Carniolan bee (*A. m. carnica*), to highly endangered subspecies, such as the Maltese honeybee (*A. m. ruttneri*), with only a few hundred colonies left [10–12]. Many of the native subspecies in Europe are currently in danger of replacement or hybridization with economically more attractive subspecies such as *A. m. carnica* or *A. m. ligustica* [12]. The Asian honeybee (*Apis cerana*) is facing similar threats [13]. While breeding activities for this species are still scarce, it is widely assumed that it can be bred in the same way as *A. mellifera*, and the first breeding efforts yielded promising results [14, 15].

Genetic improvement of local subspecies through breeding provides a great chance for their conservation, since keeping them becomes more attractive [16–18]. However, whenever breeding is practiced in small populations, the benefits of genetic improvement have to be weighed against the negative effects of increased inbreeding rates and loss of genetic variance [18–21]. The Food and Agriculture Organization of the United Nations (FAO) suggests that breeding schemes for agricultural species should not lead to an increase in inbreeding coefficients of more than 0.5% to 1% per generation [22]. This is equivalent to an effective population size of $N_e = 50$ to $N_e = 100$. These thresholds have recently been used to evaluate breeding populations in a wide range of domesticated species, including cattle [23], swine [24], sheep [25], chicken [26], horses [27], rabbits [28], dogs [29], and donkeys [30].

The development of inbreeding in a population can be steered by the selection rates for dams and sires. In most livestock species, selection rates are assessed by the (total or relative) number of selected parents [31, 32]. However, in honeybee breeding, the intensity of selection for dam queens is commonly determined indirectly via the sizes of sister groups of queens. If the population size is constant, the notions of selection rate and sister group size are reciprocal: If a relative number r of queens are selected as dams, each dam will on average have $1/r$ offspring. A likely reason for this peculiar terminology in honeybee

breeding is that, unlike in other species, breeders can easily regulate the number of queen offspring from a dam by grafting larvae [5].

In modern honeybee breeding, much pioneer work has been established by Brother Adam of Buckfast Abbey. Starting in the 1920s, he followed the approach of combining desirable properties of different subspecies by controlled hybridization. This required extensive performance tests, during which he placed numerous sister groups of colonies (i.e., colonies whose queens shared a common dam) on a variety of apiaries to obtain reliable results regarding which genetic lines proved superior. In his breeding practice, the sizes of these sister groups regularly exceeded the numbers of 20 or even 30 colonies [33]. Working with such big sister groups, however, implies that, assuming a constant population census, only 3% to 5% of all queens can be selected as dams. While this may be possible in crossbreeding schemes like those for Buckfast bees, such sharp selection can hardly be sustainable for breeding in closed populations. In 1972, the International Federation of Beekeepers' Associations (Apimondia) recommended performance tests with sister group sizes of 12 queens; i.e., a selection rate of 8.3% [34]. Since then, the introduction of best linear unbiased prediction (BLUP) breeding value estimation in the 1990s has facilitated the genetic evaluation of queens by taking more remote relationships into consideration [35, 36]. Some honeybee breeding associations have thus relaxed their recommendations to sister group sizes of six to eight queens (12.5% to 16.6% of queens selected as dams) [37, 38], while other organizations still evaluate groups of 12 sister queens [5, 39].

Not only the selection intensity on the dam's side, but also the selection of sires has an influence on honeybee populations. However, the latter is complicated by the honeybee's mating biology. Shortly after hatching, virgin queens perform one or several flights to nearby drone congregation areas where they mate with several drones from other colonies [40]. Currently, the most common ways to guarantee controlled mating of queens with valuable drone material are the use of isolated mating stations and artificial insemination [7]. On mating stations, where virgin queens are brought to perform their nuptial flights, drones are provided by colonies that are set up specifically for this purpose. By geographic remoteness, the presence of other drones can be excluded. Choosing the queens of the drone-producing colonies to share a common dam with superior breeding values achieves a high genetic standard of drones [1]. The average number of drones a queen mates with on a mating station is often assumed to be 12 [2, 40–42]. Providing controlled mating for honeybees requires considerable organization and logistics, but as shown in a previous study [43], it is paramount for successful breeding, because otherwise

the genetic gain is severely reduced. Drones are haploid and their only known purpose for a honeybee colony lies in their role during reproduction; hence, they are regularly referred to as *flying gametes* [1, 44, 45]. The collective of drone-producing queens on a mating station is seen as the analogue to *sires* in dioecious diploid animals [2, 3, 35]. The choice of how many mating stations are set up for a honeybee breeding population thus represents the absolute number of sires, and hence determines the sharpness of selection on the paternal path.

In the 1980s, computer simulations became a popular tool to evaluate honeybee breeding strategies. Moritz [46] evaluated different within-family and mass selection schemes for their implications on inbreeding and genetic progress, while Page and Laidlaw [47] introduced and examined systems of line breeding and outcrossing. However, these studies had to make vast simplifications in the relation of inbreeding and genetic progress and did not include modern techniques of genetic evaluation. In the last decades, Monte Carlo simulations for farm animals have grown more powerful in manifold respects, allowing for investigations of complex traits under modern breeding schemes on the level of individuals [48, 49]. In an earlier study, we introduced the program BeeSim, which made these concepts available for the honeybee with its biological peculiarities [3]. Now, we use it to examine a great variety of breeding schemes to study the long-term implications of different selection intensities on honeybee breeding populations of different sizes.

5.2 Methods

5.2.1 Breeding scheme

We used the program BeeSim [3] to examine breeding populations of three different sizes. The small population (S) comprised $N = 200$, the medium population (M) comprised $N = 500$, and the large population (L) comprised $N = 1000$ breeding queens per year (see Table 5.1 for a summary of the used variables). Here, the term *breeding queens* is used in the sense of *selection candidates* and as a distinction from drone-producing queens on mating stations. For each of the populations S, M, and L, we ran simulations over the course of 100 years in which we selected for a single directly (worker group) and maternally (queen) influenced trait. In each year, a BLUP breeding value estimation was carried out based on simulated performance data, and the best two-year-old breeding queens were selected to produce the next generation of breeding queens. We simulated ten different

selection rates on this path, represented by the number k_d ($1 \leq k_d \leq 10$) of queens that shared the same dam; i.e., $\lfloor N/k_d \rfloor$ dam queens were selected across families and each selected queen produced k_d daughter queens. In cases where k_d did not divide N, the remaining queens had dams that were randomly chosen among the selected two-year-old breeding queens.

Variable	**Definition**	**Values**
N	Number of breeding queens per year.	$200, 500, 1000$
k_d	Sister group size, number of queens produced by one dam.	$1, 2, ..., 10$
k_s	Percentage of breeding queens selected to produce sires.	$1, 2, ..., 10$
$S_{k_s}^{k_d}$	Selection scheme defined by key values k_d and k_s.	
N_l	Number of loci to determine a trait.	$200, 400$
$(\sigma^2_{A,m}, \sigma^2_{A,d})$	Maternal and direct additive genetic variance.	$(1, 2)$
$\sigma_{A,md}$	Genetic covariance between maternal and direct effects.	-0.75^{a}, -1.25^{b}
r_{md}	Genetic correlation between maternal and direct effects.	-0.53^{a}, -0.88^{b}
σ^2_E	Residual variance.	1
$\sigma_{A,\text{IC}}$	Genetic standard deviation, inheritance criterion.	1.22^{a}, 0.71^{b}
h^2	Total heritability.	0.79^{a}, 0.36^{b}
(h^2_m, h^2_d)	Maternal and direct heritabilities.	$(0.53, 0.34)^{\text{a}}$, $(0.72, 0.46)^{\text{b}}$
$T_{N_l}^{r_{md}}$	Trait determined by N_l loci and correlation r_{md} between effects.	$T_{200}^{-0.53}$, $T_{400}^{-0.53}$, $T_{200}^{-0.88}$, $T_{400}^{-0.88}$

[a,b] Values with the same superscript only occurred in combination; the other values could be combined freely. Values for the traits (last row) result directly from the values of N_l and r_{md}.

Table 5.1: **Variable definitions.**

All queens were brought to isolated mating stations where they mated with twelve drones each. The drones on a mating station came from eight drone-producing colonies, the queens of which shared a common dam (see [1, 35] for more detailed descriptions of honeybee mating stations). We simulated ten different selection intensities on the sires' path by different numbers of mating stations, relative to the total population; i.e., in each year, the k_s% ($1 \leq k_s \leq 10$) best three-year-old breeding queens were selected to produce the set-up of one mating station each.

The different choices of k_d and k_s stood for different selection intensities on the maternal and paternal paths, respectively. In combination, they defined 100 distinct selection schemes for each of the populations S, M, and L. For given k_d and k_s, we named the corresponding breeding scheme $S_{k_s}^{k_d}$.

To facilitate the interpretation of the simulation results, we give a general idea of how k_d and k_s influence genetic gain and inbreeding in quantitative genetic theory—particularly because the sister group size k_d is a non-common variable in this context. Selection response depends linearly on the selection intensity i, which in turn can be calculated from the selection rates k_s on the sire's path and $1/k_d$ on the dam's path. Note in particular that intensified selection corresponds to higher values of k_d but lower values of k_s. Inbreeding rates depend on absolute numbers of selected dams and sires rather than selection rates. Making vast simplifications, Wright [50] estimated the inbreeding rate in a population with M reproducing males and F reproducing females as $\Delta F \approx (F + M)/8MF$. While this formula should not be directly applied to honeybees, it still signifies that inbreeding rates are mainly determined by the total numbers N/k_d of dams and $N \cdot k_s$ of sires.

5.2.2 Genetic models

The queens' genetics were simulated based on a finite locus model with $N_l = 200$ or $N_l = 400$ unlinked loci and were inherited according to the Mendelian rules without mutations, as described in [3]. All selection traits had an initial maternal additive variance of $\sigma^2_{A,m} = 1$, an initial direct additive variance of $\sigma^2_{A,d} = 2$, and a residual variance of $\sigma^2_E = 1$. Two different values were chosen for the covariance between maternal and direct effects: A moderately negative covariance between maternal and direct effects of $\sigma_{A,md} = -0.75$ and a strongly negative covariance of $\sigma_{A,md} = -1.25$ were considered. The resulting total genetic standard deviations in the sum of maternal and direct breeding values of queens (the inheritance criterion) were $\sigma_{A,\mathrm{IC}} = 1.22$ and $\sigma_{A,\mathrm{IC}} = 0.71$. The setting with moderate negative correlation ($\sigma_{A,md} = -0.75$) reflected initial maternal, direct, and total heritabilities of $h^2_m = 0.53$, $h^2_d = 0.34$, and $h^2 = 0.79$, respectively. The correlation between both effects was $r_{md} = -0.53$. The corresponding values for the traits with strong negative correlation ($\sigma_{A,md} = -1.25$) were $h^2_m = 0.72$, $h^2_d = 0.46$, $h^2 = 0.36$, and $r_{md} = -0.88$ (heritabilities calculated according to Brascamp and Bijma [42]). These parameters have previously been used in honeybee-specific breeding simulations [3, 43] and are in accordance with what has been estimated for European honeybee populations for economically relevant traits, such as honey yield and gentleness [41, 51]. The set-up of individual loci of the queens, workers, and drones from the base population as well as the inheritance of alleles to later generations were performed by the BeeSim software [3]. All loci were simulated as purely additive and biallelic, while allele frequencies followed a U-shaped $\beta(0.5, 0.5)$-distribution. Allele effects were sampled following a mixture of multivariate

Laplace and Normal distribution with variance $\mathbf{\Sigma}_A = \begin{bmatrix} \sigma^2_{A,m} & \sigma_{A,md} \\ \sigma_{A,md} & \sigma^2_{A,d} \end{bmatrix}$ and post-corrected to yield the desired genetic variances exactly. This procedure was identical to that used in [3], where it is described in greater detail. The colonies' performance records were generated as the sum of their queens' maternal breeding values, their worker groups' direct breeding values, and residual values with variance σ^2_E. All fixed environmental effects were modeled to be zero.

The different combinations of the numbers of loci ($N_l = 200$ or $N_l = 400$) and initial negative correlations of maternal and direct effects ($r_{md} = -0.53$ or $r_{md} = -0.88$) led to four distinct selection traits, which we refer to as $T^{-0.53}_{200}$, $T^{-0.53}_{400}$, $T^{-0.88}_{200}$, and $T^{-0.88}_{400}$, respectively.

For each of the 1200 combinations of population size (S, M, L), selection scheme ($S^{k_d}_{k_s}$, $1 \leq k_d, k_s \leq 10$) and trait ($T^{-0.53}_{200}$, $T^{-0.53}_{400}$, $T^{-0.88}_{200}$, $T^{-0.88}_{400}$), simulations were carried out with 24 repetitions in order to obtain stable results.

5.2.3 Breeding value estimation

Based on the simulated performance tests, we carried out a BLUP breeding value estimation using the BLUPF90 software [52]. The inverse numerator relationship matrix was calculated with a bee specific approach, as it is described by Bernstein et al. [2], following the ideas of Brascamp and Bijma [1]. Each of the simulated colonies was assigned to one of $7N/100$ apiaries, leading to an average apiary size of 14.3 colonies. In the breeding value estimation, each combination of year and apiary was considered as a fixed effect. The queens of the base population were assigned to the apiaries randomly. In later generations, 70% of queens were assigned the same apiary as their dam, while the remainder were assigned to random apiaries. This steadiness in apiaries resembles that of the Central European breeding population represented by beebreed.eu [53].

In the first years of selection, the previously defined exact genetic parameters were used as inputs for the breeding value estimation. However, in the finite locus model, the allele frequencies within a population under selection shift over time due to selection and drift processes. Thus, a change in genetic parameters can be observed over time, which leads to biased results of the breeding value estimation if it is not accounted for [3].

We therefore reevaluated the genetic parameters that were used for the BLUP breeding value estimation every five years. We did so based on the within-family variance, to avoid biased results due to the Bulmer effect [54]. Following ([3], Equation (7)), the variance of

the Mendelian sampling, $\boldsymbol{\delta}_D = \begin{bmatrix} \delta_D^{\text{mat}} \\ \delta_D^{\text{dir}} \end{bmatrix}$, in the inheritance from a queen Q with inbreeding coefficient F_Q to a drone D can be estimated as

$$\text{var}(\boldsymbol{\delta}_D) = (1 - F_Q) \cdot \boldsymbol{\Sigma}_A.$$

Drones are hereby interpreted as diploid but homozygous at all loci, as it is sometimes assumed in theoretical honeybee genetics [3, 55]. In every fifth year, we let all breeding queens $(Q_1, ..., Q_N)$ of that year produce 100 drones $(D_{Q_i,1}, ..., D_{Q_i,100})$ each. With

$$\boldsymbol{\delta}_{D_{Q_i,j}} := \mathbf{TBV}(Q_i) - \mathbf{TBV}(D_{Q_i,j}),$$

we then calculated the additive genetic variance in that year as

$$\hat{\boldsymbol{\Sigma}}_A = \frac{1}{100N - 1} \sum_{i=1}^{N} \frac{1}{1 - F_{Q_i}} \sum_{j=1}^{100} \boldsymbol{\delta}_{D_{Q_i,j}} \boldsymbol{\delta}'_{D_{Q_i,j}}.$$

For this and the following four years, the breeding value estimation was performed with the use of the newly estimated $\hat{\boldsymbol{\Sigma}}_A$. Although genetic parameters changed over time, BLUP was always performed with the full pedigree and all historical performance records, as is the current practice in the European breeding programs represented by beebreed.eu [53].

5.2.4 Analysis of simulation output

The analysis of genetic changes in the populations focused on three key values: genetic gain, genetic variance, and generational rate of inbreeding. While genetic gain is the main incentive for breeding, the development of genetic variance and inbreeding signify the sustainability of breeding programs. The genetic gain in a specified year was measured as the average sum of maternal breeding values of queens and direct breeding values of worker groups. This so-called *performance criterion* signifies the average phenotypic superiority over the base population [3]. Genetic variance was measured in the so-called *inheritance criterion*; i.e., we considered the variance of the sum of maternal and direct breeding values of queens. Since only queens are able to reproduce, this value reflects the population's potential for further genetic change [42]. The generational inbreeding rate ΔF is defined as $\Delta F = \frac{F_{t+1} - F_t}{1 - F_t}$, where F_t and F_{t+1} are average inbreeding coefficients in successive generations, and is usually assumed to be constant over generations. Consequently, we calculated ΔF as $\Delta F = 1 - (1 - F_{100})^{2.5/99}$, where F_{100} is the average inbreeding coefficient

of queens in year 100, 99 is the number of years elapsed since year 1, and 2.5 is the average generation interval (two years on the maternal path and three years on the paternal path). Individual inbreeding coefficients were calculated from the pedigree. All analyses were carried out using the statistical software R [56].

5.3 Results

5.3.1 Overview of the simulation outcome

The genetic gain after 100 years varied between 10.80 and 19.15 units in simulations with $r_{md} = -0.53$ and between 3.85 and 9.90 units in simulations with $r_{md} = -0.88$. The 24 repetitions of the simulations revealed an average standard deviation in genetic gain after 100 years of 0.54 units. Genetic gain in population L (N=1000) was on average 0.76 units higher than in population M ($N = 500$) and 2.09 units higher than in population S ($N = 200$). Traits that were determined by 400 loci yielded on average 1.89 units higher genetic gain (see Figure 5.1A). The effects of population size and number of loci on genetic gain were only substantial in the long term; after 15 years there were only marginal differences (see Figure 5.1B). Generational inbreeding rates ranged between $\Delta F = 0.06\%$ and $\Delta F = 2.59\%$. Larger populations showed lower inbreeding rates, while the number of loci with influence on the trait had no effect. The traits with strong negative correlation between queen and worker effects, $r_{md} = -0.88$, yielded higher generational inbreeding rates than the traits with moderate correlation, $r_{md} = -0.53$ (see Figure 5.1C). Comparing the generational inbreeding rates with the thresholds suggested by the FAO, we found that in the small population ($N = 200$), 21.25% of all combinations of breeding schemes and traits resulted in a generational inbreeding rate $\leq 1\%$ and 6% of the combinations yielded a generational inbreeding rate $\leq 0.5\%$. In the larger population, the corresponding percentages were 54.75% and 19% ($N = 500$), and 82.5% and 39.75% ($N = 1000$). The standard deviation of inbreeding rates over the 24 repetitions was 0.08%. When we compared the additive genetic population variance after 100 years with the variance in the base population, we found that on average, 21.74% of the initial variance was maintained (see Figure 5.1D). Among the schemes with generational inbreeding rates below 1%, the maintenance rate of genetic variance was 27.05% (35.49% for $\Delta F \leq 0.5\%$).

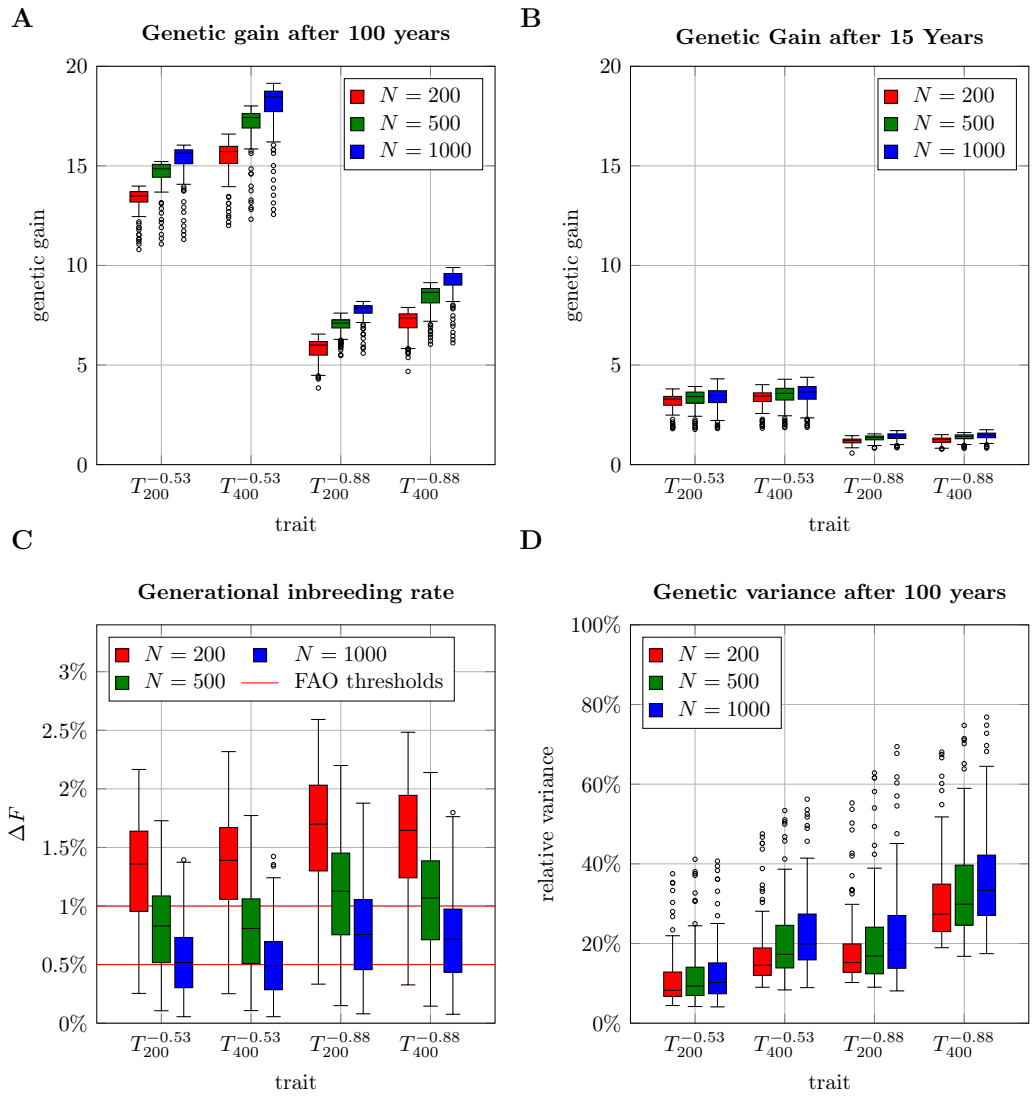

Figure 5.1: **General results.** Simulated results for genetic gain after 100 (**A**) and after 15 years (**B**), generational inbreeding rates (**C**), and remaining genetic variance after 100 years (**D**). Individual boxplots are drawn for combinations of population sizes ($N = 200$, $N = 500$, $N = 1000$) and selection traits ($T_{200}^{-0.53}$, $T_{400}^{-0.53}$, $T_{200}^{-0.88}$, $T_{400}^{-0.88}$), signifying different numbers of influential loci ($N_l = 200, 400$) and correlations between maternal and direct effects ($r_{md} = -0.53, -0.88$). The boxplots comprise the results obtained for varying sister group sizes ($1 \leq k_d \leq 10$) and selection rates for sires ($1 \leq k_s \leq 10$).

5.3.2 Comparison of breeding schemes

Generational inbreeding rates

In all populations and for all traits, schemes with sharper selection resulted in higher generational inbreeding rates; i.e., generational inbreeding rates increased with the sizes of queen sister groups, k_d, and decreased with higher numbers of sires (see Figure 5.2 for a typical example). While for large numbers of mating stations the generational inbreeding rates depended nearly linearly on the sizes of sister groups, the dependency was slightly sublinear for small numbers of mating stations. Depending on the number of mating stations, an increase of sister group sizes by one queen caused average increases in the generational inbreeding rate between 0.09% and 0.16% for $N = 200$, between 0.09% and 0.15% for $N = 500$, and between 0.06% and 0.13% for $N = 1000$ (values in absolute percentage points). When it came to the influence of the number of sires, we found a convex dependency; i.e., when the total number of sires was low, the addition of further sires decreased the generational inbreeding rates more than when the number of sires was high. Stepping from $k_s = 1$ to $k_s = 2$ decreased the generational inbreeding rate on average by 0.29%, while the step from $k_s = 9$ to $k_s = 10$ only yielded an average decrease by 0.04% (absolute percent points).

Sustainable genetic gain

Looking at the genetic gain after 100 years of selection, we observed two opposing effects influencing the outcomes. A strong selection led to high rates of genetic gain in the first years but also caused severe reduction of genetic variance which diminished the gain rates in later years (see Figure 5.3 for examples). As a result, when comparing accumulated genetic response after 100 years with generational inbreeding rates, we found inverted-U shaped connections in all of the simulations (see Figure 5.4). In larger populations, the maximum rates of genetic gain were reached at lower generational inbreeding rates than in smaller populations.

We considered a breeding scheme to be competitive in the 100 year frame if the genetic gain after 100 years was not more than 5% lower than in the breeding scheme with the highest genetic gain. Table 5.2 gives an overview of the competitive breeding schemes (CBSs) in the different scenarios. When there was a strong negative correlation between maternal and direct effects ($r_{md} = -0.88$), there were significantly fewer CBSs than for the moderate correlation ($r_{md} = -0.53$). Furthermore, larger populations allowed for

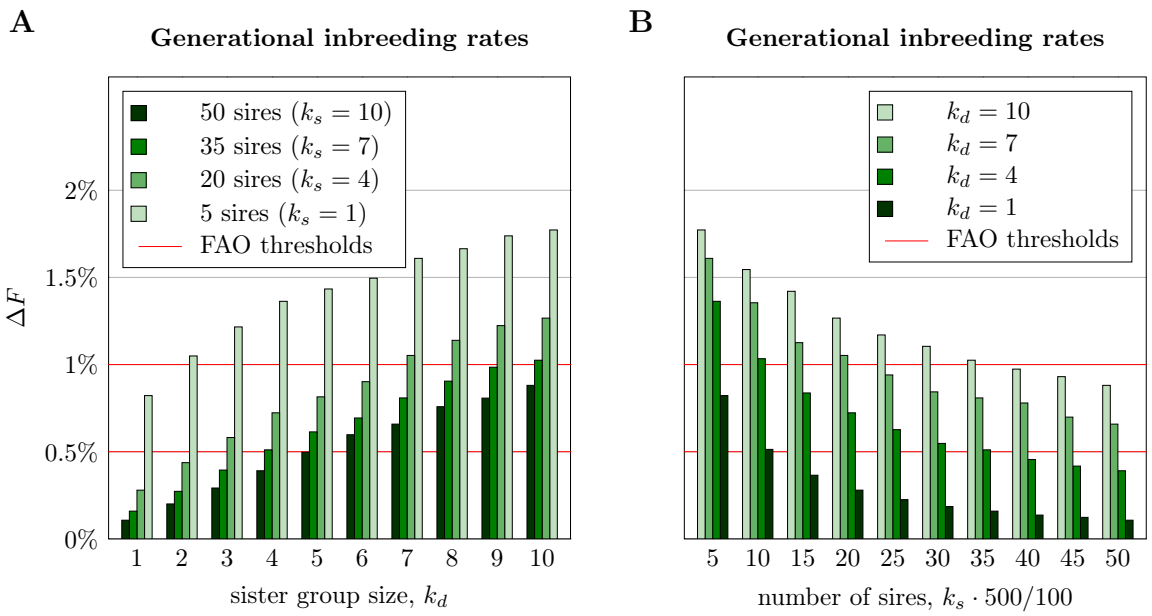

Figure 5.2: **Inbreeding rates under different selection intensities.** Generational inbreeding rates for different selection schemes on a population of $N = 500$ colonies per year for trait $T_{400}^{-0.53}$ with moderate negative correlation between maternal and direct effects ($r_{md} = -0.53$), influenced by 400 loci. The subfigures show the influences of different sister group sizes (**A**) and numbers of sires (**B**).

more CBSs than smaller ones. All combinations of trait and population size allowed for CBSs with generational inbreeding rates below 1%. However, the stricter threshold of $\Delta F \leq 0.5\%$ was only kept in the larger populations ($N = 500$, $N = 1000$) and mostly for the traits with moderate correlation between effects ($r_{md} = -0.53$).

In the small population ($N = 200$), most breeding schemes which turned out both competitive and sustainable ($\Delta F \leq 1\%$) featured sister group sizes of $k_d = 3$ or $k_d = 4$. The number of mating stations in these breeding schemes was generally high; k_s was mostly ≥ 6.

In population M ($N = 500$), most sustainable CBSs had sister group sizes of $k_d \geq 4$ and the numbers of sires were mostly determined by values of $k_s \geq 4$. Stronger selection of dams, i.e., larger sister group sizes, required higher numbers of mating stations to remain sustainable. When the number of mating stations was very high ($k_s \geq 9$), the traits with medium correlation between effects ($r_{md} = -0.53$) even allowed for sustainable selection with the largest size of sister groups ($k_d = 10$). For the other traits ($r_{md} = -0.88$), the maximum sister group size that still allowed for sustainable breeding was $k_d = 7$, but

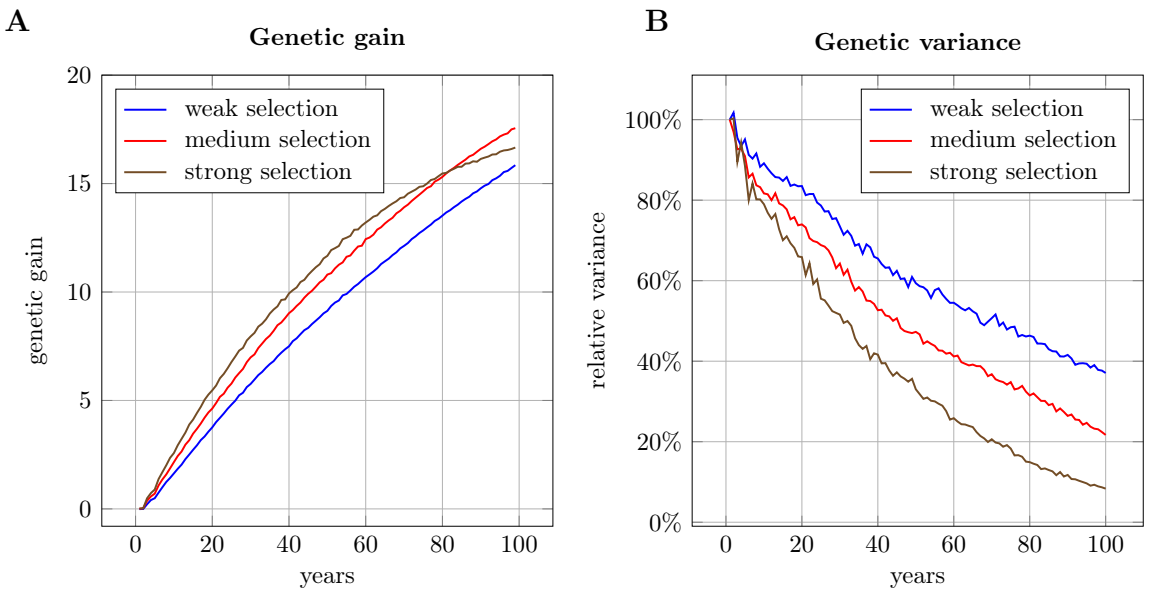

Figure 5.3: **Genetic gain and genetic variance.** Development of genetic gain (**A**) and genetic variance (**B**) over time for three selection intensities: strong selection ($k_d = 9$, $k_s = 1$), medium selection ($k_d = 4$, $k_s = 6$), and weak selection ($k_d = 2$, $k_s = 9$). Initially, intense selection yields higher rates of genetic gain, but the severe reduction of genetic variance leads to reduced gain in later years. Graphs are shown for a population of 500 queens per year and selection for trait $T_{400}^{-0.53}$ with 400 influential loci and moderate correlation between effects ($r_{md} = -0.53$).

only when there were many sires for the queens to mate with ($k_s \geq 9$). The few CBSs in population M with generational inbreeding rates below the stricter threshold of 0.5% mostly had sister group sizes of $k_d = 4$ and large numbers of sires ($k_s \geq 8$).

In the large population ($N = 1000$), 20.5% of all CBSs passed the strict sustainability criterion of $\Delta F \leq 0.5\%$. For these schemes, the sister group size k_d mostly lay between 4 and 6 and the number of sires was determined by values of $k_s \geq 4$. Trait $T_{400}^{-0.88}$ only allowed for CBSs with the weaker sustainability criterion $\Delta F \leq 1\%$. The CBSs that fulfilled this criterion featured sister group sizes of five or more queens. Here, large numbers of sires required a sharp selection on the dam's path in order to stay competitive. For $k_s = 10$, only breeding schemes with sister group sizes of $k_d \geq 8$ were competitive.

All CBSs experienced severe losses of more than 63% in genetic variance over the course of 100 years. Losses were worst for trait $T_{200}^{-0.53}$, where even the competitive breeding schemes with the lowest generational inbreeding rates lost over 85% of the initial genetic variance in the population.

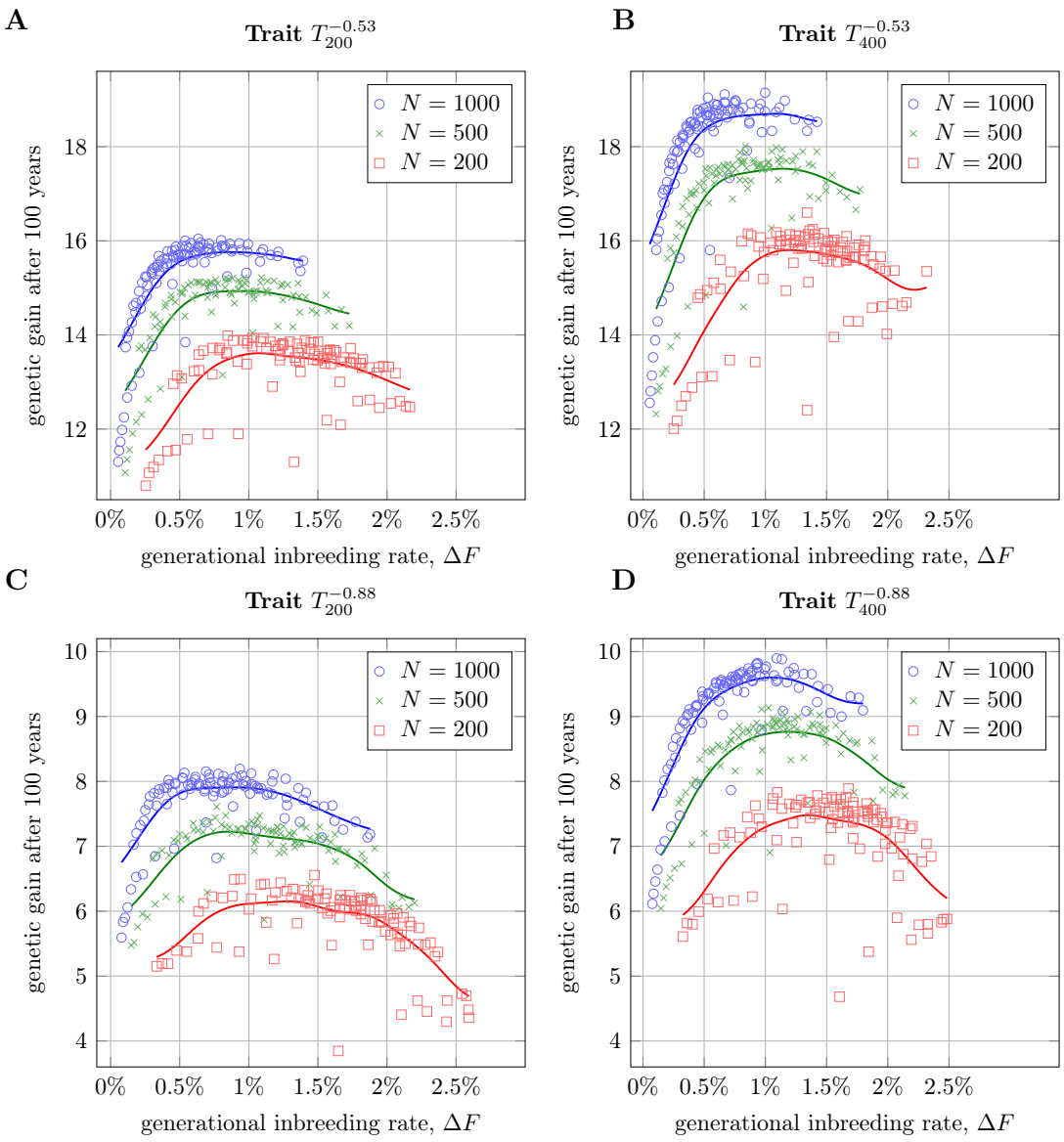

Figure 5.4: **Inbreeding rates and genetic response.** Scatter-plot of genetic response after 100 years vs. generational inbreeding rate. The regression curves were obtained using a Gaussian kernel with bandwidth 0.003, as implemented in the R package `np` [56, 57]. Subfigures (**A**) to (**D**) represent the four simulated traits, defined by number of influential loci ($N_l = 200, 400$) and the correlation between maternal and direct effects ($r_{md} = -0.53, -0.88$).

5.3.3 Short-term genetic gain

The simulated scope of 100 years lies far outside the planning range of practical beekeepers or animal breeders in general. To evaluate the breeding schemes for attractiveness for

					CBS with lowest ΔF				CBS with highest ΔF			
		N_{CBS}[a]	$N_{1\%}$[b]	$N_{0.5\%}$[b]	k_d	k_s	ΔF	var[c]	k_d	k_s	ΔF	var[c]
$N = 200$	$T_{200}^{-0.53}$	70	14	0	3	10	0.64%	16.89%	10	3	2.01%	6.34%
	$T_{400}^{-0.53}$	48	5	0	4	10	0.81%	21.67%	10	5	1.82%	12.80%
	$T_{200}^{-0.88}$	16	3	0	2	6	0.88%	28.26%	8	8	1.73%	10.24%
	$T_{400}^{-0.88}$	37	1	0	3	9	0.92%	36.76%	7	4	1.94%	15.80%
$N = 500$	$T_{200}^{-0.53}$	74	46	5	3	7	0.39%	15.45%	9	1	1.67%	5.29%
	$T_{400}^{-0.53}$	64	42	2	4	8	0.46%	25.10%	10	2	1.55%	12.59%
	$T_{200}^{-0.88}$	32	17	0	4	10	0.60%	27.34%	9	5	1.51%	8.49%
	$T_{400}^{-0.88}$	47	11	0	5	9	0.74%	37.22%	9	3	1.64%	15.66%
$N = 1000$	$T_{200}^{-0.53}$	73	64	23	4	9	0.25%	15.13%	10	1	1.39%	5.15%
	$T_{400}^{-0.53}$	64	56	18	5	8	0.32%	25.06%	10	1	1.42%	11.02%
	$T_{200}^{-0.88}$	63	48	10	3	8	0.32%	34.02%	10	3	1.41%	7.29%
	$T_{400}^{-0.88}$	49	34	0	6	8	0.58%	35.46%	10	2	1.51%	13.35%

[a] Number of competitive breeding schemes

[b] Number of competitive breeding schemes with generational inbreeding rate $\Delta F \leq 1\%$, resp. $\Delta F \leq 0.5\%$

[c] Genetic variance in year 100 relative to the genetic variance in the base population

Table 5.2: **Competitive breeding schemes (CBS) after 100 years.**

honeybee breeders, we therefore also investigated genetic gain after 15 years of breeding. Here the breeding success depended mostly on the breeding scheme and the genetic correlation between effects but not so much on the number of involved loci or the population size (see Figure 5.1B). We found that sharper selection on the dam's path, i.e., larger sister group sizes, led to greater response to selection (see Figure 5.5A). A similar influence was observed for the number of sires in the traits with medium correlation between effects ($r_{md} = -0.53$). For the traits with strongly negative correlation ($r_{md} = -0.88$), however, there was no clear directional impact of the number of mating stations on the genetic gain after 15 years (Figure 5.5B).

The genetic gain after 15 years, dependent on the sister group size k_d of breeding queens, followed the law of diminishing marginal utility; i.e., the larger the sister group size was, the smaller the additional genetic gain that was achieved by further increasing the sister group size. Compared to a sister group size of $k_d = 10$, which on average yielded the highest genetic gain after 15 years, the genetic gain was reduced by 4.92% for $k_d = 6$, by 10.03% for $k_d = 4$, and by 14.17% for $k_d = 3$.

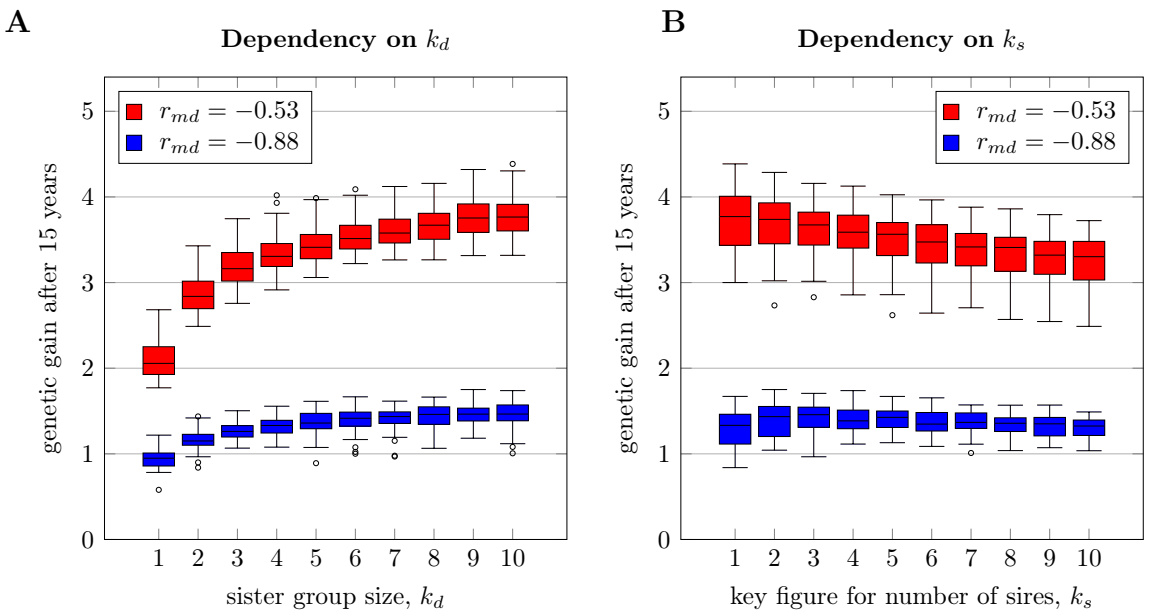

Figure 5.5: **Genetic gain after 15 years.** Box plots showing the average genetic gain after 15 years for different sister group sizes (**A**) and different numbers of sires (**B**). In part (**B**), values for sister group size $k_d = 1$ are omitted to avoid an excess of outliers.

The number of sires, represented by k_s, had a smaller effect on the genetic gain after 15 years than decisions on the dam selecting path. In the traits with moderate genetic correlation ($r_{md} = -0.53$), every increment of k_s by one caused an average reduction in genetic gain by 0.92% in the small population S, by 1.64% in the medium population M, and by 2.03% in the large population L. When the genetic correlation was stronger ($r_{md} = -0.88$), the average genetic gain after 15 years was highest for $k_s = 3$ with 1.39 units and ranged lowest for $k_s = 10$ at 1.25 units (9.64% lower).

5.4 Discussion

5.4.1 Reality check on assumptions

Our simulation study suggests that sustainable breeding for honeybees is generally possible, even for populations consisting of as few as 200 colonies per year. However, as for any mathematical model of biological processes, our analysis is based on assumptions. In the following, we discuss the plausibility of the most crucial premises of this study.

Population structure

Our simulated populations had a very homogeneous structure. Every year comprised the same numbers of breeding colonies, selected queens, and mating stations. Furthermore, all queens were selected at constant rates for the same single trait and strict truncation selection was applied. Real populations are much more diverse. Because breeders are usually organized locally, a significant amount of genetic transfer occurs locally, leading to clustered populations with partly connected sub-populations which mix only to a limited extent. In comparison with the simulated homogeneous population, clustered populations have higher inbreeding rates and thus smaller effective population sizes [58]. In places with unfavorable environmental conditions, the population size may reduce drastically in some years, if only few colonies are overwintered successfully [59]. The occurrence of such population bottlenecks may further increase inbreeding rates [60]. However, there are also factors which reduce inbreeding in reality compared to our study. Most notably, the assumption of truncation selection across families is unlikely to be followed strictly. While offspring of the queens with the absolute highest estimated breeding values will certainly be coveted, some breeders will also select primarily among the offspring of their own queens. The result is likely to be a hybrid scheme between across-family and within-family selection. Similar selection rates generally yield lower rates of inbreeding when within-family selection is applied [61]; Moritz [46] explicitly confirmed this for the honeybee. Moreover, selection decisions are further diversified by different breeding foci of individual breeders, following a variety of trade-off decisions between more than one trait [62]. Furthermore, selection foci may shift over time because of environmental changes or technical innovations. Taking both parts into consideration, we conclude that while our simulation studies inevitably are imperfect reflections of real honeybee breeding, we do not have any evidence that our results are thereby biased in a specific direction.

Genetic model

Our simulations used a genetic model with a finite number of unlinked loci, as is recommended by Plate et al. [3] for long-term studies. The argument made for the finite locus model in this previous study is that in comparison with the infinitesimal model, it is a more accurate reflection of biological reality and that it minimizes the risk of underestimating the loss of genetic variance due to drift and selection. However, Hill [63] argues that finite locus models actually exaggerate the extent of decrease of genetic vari-

ance. Furthermore, we did not model any non-additive effects which may have a positive influence on the maintenance of genetic variance [64]. The severe losses we observed in this study might therefore draw an overly dramatic picture. Partly because of this reason, we decided to focus our analysis mainly on generational inbreeding rates rather than maintenance of genetic variance, because inbreeding rates depend less on the choice of the genetic model [3]. We included neither linkage nor mutations into our model. While linkage has been shown to be of little effect in the framework of this study [3], mutations could potentially help to retain genetic variance over the long period of 100 years [65].

Analysis of results

Our analyses relied on 24 repetitions of a Monte Carlo process. The standard deviations for the results in genetic gain and inbreeding were small in comparison to their absolute values; hence, we obtained stable results. This is in line with an earlier study [3] that showed that reliable results in breeding simulations for honeybees can be achieved with relatively few repetitions.

We based a large part of our analysis on the assumption that sustainable breeding is possible if the generational inbreeding rate is lower than 0.5% to 1% per generation, as is recommended by the FAO. These thresholds are mainly based on theoretical works by Franklin [66] and Soulé [67]. As pointed out by Shaffer [68], recommendations of this kind should be regarded as "rough guidelines rather than specific prescriptions." Because breeding depends on several random processes, an individual realization of a breeding scheme may produce higher inbreeding rates than predicted by our simulations. But since the 24 replications of our simulations revealed only small standard deviations of generational inbreeding rates (0.08%), this risk appears negligible. The FAO thresholds have occasionally been criticized as too high by conservation biologists [69, 70], but are widely thought of as plausible in animal breeding [71]. For honeybees, it has been shown that inbreeding depression has negative influences on traits such as honey yield and swarming behavior [72, 73]. However, these studies do not give reliable estimates of which inbreeding rates allow for sustainable breeding of honeybees. Compared to other species, in honeybees, there is an additional way in which inbreeding has negative effects on the colony; namely, the genetics at the sex-determining locus *csd*. Worker brood that is homozygous at this locus is removed from the hive, leaving gaps in the brood and thus acting negatively on the colony size [74]. Zayed [75] thus argues that honeybees are especially vulnerable to inbreeding and special care has to be taken. Contrary to this

effect, inbreeding depression due to homozygosity of recessive deleterious alleles at other loci is likely to be expressed in haploid drones directly. The mating procedure may thus implicitly select against such alleles and alleviate consequences of inbreeding [76]. We are not aware of any dependable data that support a claim that generational inbreeding rates in honeybee breeding programs should obey different thresholds than those in selection programs of other livestock.

We introduced the notion of a competitive breeding scheme (CBS) to judge breeding decisions in a long-term frame. Breeding schemes were considered competitive if genetic gain after 100 years was reduced by at most 5% in comparison to the most successful scheme. On average, these 5% roughly represented the standard deviations obtained from the 24 repetitions. Therefore, this definition compensates for the fact that individual realizations of breeding schemes may vary in their results, while still allowing one to distinguish the most successful breeding schemes. We think of it as a valuable tool in the assessment of breeding schemes. However, it should only be used in combination with other criteria that provide a direct evaluation of inbreeding or genetic variance. Table 5.2 and Figure 5.4 show that, particularly in small populations, breeding schemes can be evaluated as "competitive" while accumulating high inbreeding rates.

The time point after 100 years to evaluate the long-term effects was to some extent an arbitrary choice. Figure 5.3 shows that even with intense selection, there is still some, albeit reduced genetic progress in the last years simulated. Had the evaluation time been chosen to be later, it is likely that fewer of the unsustainable breeding schemes would have appeared to be competitive. Under simplifying assumptions, Dempster [77] and Robertson [78] showed that under indefinite selection, the highest ultimate genetic gain is to be expected if about half of all individuals are selected as parents in each generation. This corresponds to lower selection rates than in any breeding scheme considered competitive in the present study.

5.4.2 Influences of simulation parameters

Population size

Population size proved to be a key factor that is to be considered in the design of breeding strategies. Larger populations proved superior in regard to genetic gain, conservation of genetic variance, and avoidance of inbreeding (Figure 5.1). Furthermore, relations between various influencing factors can be determined more clearly in larger populations; e.g., the

connection between inbreeding and genetic gain in Figure 5.4 appears more scattered for the smaller populations. This is due in part to averaging effects leading to less variable simulation results in larger populations [3], and in part to the fact that inbreeding effects are more punishing in smaller populations since favorable alleles can be lost more easily. The present simulations included population sizes of up to 1000 breeding queens per year. This is eight times smaller than the size of the Central European breeding population of *A. m. carnica* that is registered in beebreed.eu [53]. However, a single repetition of a simulation of a breeding scheme for a specific trait in the large population had a runtime of approximately nine hours on the high performance computing cluster of the North-German Supercomputing Alliance (HLRN) and the simulation time depended clearly superlinearly on the population size. The scope of the study presented here with 24 repetitions for 1200 settings was only possible due to massive parallelization efforts. Running simulations for populations of a size like that in beebreed.eu thus seems currently out of reach. The beebreed.eu population has strong regional relationship clusters, which, as described above, reduce its effective population size. Extrapolations from the simulated data are therefore difficult and prone to imprecision. But our simulations showed that larger population sizes are also more forgiving when it comes to breeding decisions, having higher total numbers of competitive breeding schemes. Therefore, we are confident that good breeding results can be achieved for populations of the size of Central European *A. m. carnica* if one follows a rough guideline that selection can be slightly more intense than in the simulated population L, consisting of 1000 colonies per year.

The simulations also do not cover very small populations of less than 200 queens per year. One can argue that sustainable breeding for such populations will require even lower sister group sizes than the smallest simulated population, S, and that a higher proportional number of sires will be necessary. However, as the effective population sizes for sustainable breeding should not drop below $N_e = 50$ to $N_e = 100$, these numbers also form strict lower bounds on the actual population size. Moritz [46] argues that breeding of very small populations should only be applied over few generations. In recent years, great advances have been achieved in cryopreservation methods for the honeybee [79], potentially opening the door for sustainable breeding for very small populations [80]. However, the use of preserved semen or embryos would lead to extended generation intervals, and therefore a slow-down of the genetic progress.

Genetic parameters

Negative correlations between effects complicated breeding efforts, leading to lower genetic gain and increased generational inbreeding rates (see Figure 5.1). Both effects have previously been observed in simulations on honeybees [3, 43] and in more general settings of animal breeding [81, 82]. The increased generational inbreeding rates are ultimately an effect of the reduced total heritability under negative correlation between effects [83]. Higher generational inbreeding rates in similar selection schemes resulted in a more pronounced inverted-U shape in the relation between inbreeding and genetic gain (Figure 5.4) and thus fewer competitive breeding schemes. Thus, when breeding is targeted at a trait for which a strong negative correlation between effects is known, sister group sizes should be reduced and/or the number of sires should be increased in comparison to traits with less pronounced correlations. Similar measures should be taken in cases wherein one knows that a trait is influenced by only few loci, because in this case, similar rates of inbreeding translate to more severe losses of genetic variance. In practice, however, we suspect it to be difficult to customize breeding schemes to the genetic parameters of selection traits. Especially in small populations, the estimation of genetic parameters is often difficult and likely to be imprecise [39, 41]. Moreover, most breeding efforts include a variety of selection traits with different genetic parameters.

Sister group sizes

By implementing sister group sizes between $k_d = 1$ to $k_d = 10$, we covered a wide range of selection rates on the dam's path: between 10% and 100%. This signifies that there is great potential to shape selection schemes via this parameter. Our simulations suggest that in many settings for honeybee breeding it is a favorable decision in the long term to follow breeding schemes with sister group sizes k_d between three and six queens (selection rates between 17% and 33%), while the ideal number rises with the population size. On this base, the official recommendations of sister group sizes of $k_d = 6$ to $k_d = 8$ [37, 38] seem reasonable for the large Central European population of *A. m. carnica* but should not simply be transferred to smaller populations. Recommendations of large sister group sizes of twelve or more queens should be seen skeptically. However, one has to take into consideration that our simulations reflect populations that remain closed for many years. We can therefore give no clear suggestions for combination breeding schemes, such as that of Buckfast bees, which rely substantially on a steady influx of genetic material

from various subspecies [84]. Pure-bred populations are not necessarily completely closed either; for an Austrian breeding population of *A. m. carnica*, managed by the bee breeders' organization Biene Österreich, it has been reported that each year about a quarter of the colonies are newly introduced with an unregistered dam or sire [41]. Introducing unrelated colonies to the breeding population will decrease inbreeding rates but potentially hamper genetic gain. In the first years after bringing new colonies into the population it might thus be advisable to breed with larger sister group sizes to increase the accuracy of breeding value estimation [85]. If the breeding population can be enlarged early on through the addition of further colonies, this will in the long run have positive effects on the genetic gain [86]. However, for the most endangered subspecies, such as *A. m. ruttneri* and *A. m. siciliana*, the introduction of new colonies is not an option, because breeding population and total population coincide.

The use of smaller sister groups in smaller populations comes with a higher workload—because more colonies have to be prepared for queen rearing [5]—and smaller genetic gain in the short term (Figure 5.5). However, one may assume that beekeepers who are willing to work with endangered honeybee subspecies are committed to ideals beyond pure profit maximization. A 2010 study found a wide range of motivations of European cattle breeders to work with local breeds, including tradition, regional adaptation, and conservation efforts [87]. With a sister group size of $k_d = 4$, one can expect to still reach almost 90% of the maximum genetic gain after 15 years; we are thus optimistic that breeders can be convinced to follow such sustainable schemes.

If local honeybee breeding proves successful, it is likely to attract more breeders and thus yield a growing breeding population [17]. The then larger breeding populations will enable more intense selection schemes in the future.

Number of sires

In our simulations, selection for dams of sires was generally stricter than selection for dams of breeding queens, which reflects reality in honeybee breeding. Limited availability and high maintenance costs for isolated mating stations are the main reasons for the stricter selection on the sires' path in practice [5, 88]. The selection rate for sires had a lower influence on the short-term genetic gain than the selection rate for dams (Figure 5.5). This is in line with previous simulation studies on honeybees [3, 43].

For the traits with strongly negatively correlated maternal and direct effects, intensified selection rates for sires sometimes translated to lower selection intensities (see Figure 5.5B).

The reason is that for these traits, the accuracy of estimated breeding values declines with the number of mating stations [43]. Regarding optimal numbers of mating stations, we observed that while the small population, S, required high relative numbers of sires for sustainable breeding (signified by large values for k_s), an oversupply of sires proved disadvantageous to long-term genetic gain in the large population, L. This is in line with the general fact that inbreeding rates, which we took as measurement for sustainability, depend mainly on the absolute numbers of selected parents. Based on numbers of sires in existing breeding populations, we do not see an abundance of sires as a realistic threat in practical breeding with large populations [53]. On the contrary, many breeding organisations for small populations will face difficulties to provide a sufficient number of mating stations, given the limited availability of geographic places with suitable conditions [88]. Therefore, alternatives to classical mating stations should be promoted, the most important of which is probably artificial insemination of queens [89]. When inseminating queens instrumentally, breeders can mimic the situation on mating stations; i.e., use the sperm of drones from a sister group of drone-producing colonies to fertilize the queens [5, 7]. But a wide range of alternative procedures is possible, reaching from the use of mixed semen from large numbers of drones [90] to inseminations with drones from a single colony [91] or even single-drone inseminations [92]. In his simulation studies, Moritz [46] propagated artificial insemination with mixed sperm of many drones. In comparison to mating stations, these schemes yield higher genetic diversity within worker groups but not necessarily between individual queens. Furthermore, they do not allow to include paternal inheritance into the BLUP breeding value estimation and thus are not fully compatible with modern strategies of genetic evaluation. Ultimately, the long-term influences of different insemination practices on generational inbreeding rates, genetic gain, and genetic variance are yet to be investigated.

Whereas sister group sizes can easily be established by individual breeders, the design of the sires' part of breeding schemes are mainly determined by breeding organizations. Burgeoning honeybee breeding efforts in Europe and potential breeding of *Apis cerana* in Asia can only be successful in the long run if breeding organizations manage to provide a sufficient number of mating stations and/or adequate training for instrumental insemination.

5.4.3 Further breeding schemes

The breeding schemes of Page and Laidlaw [47] build on several breeding lines that are kept separated for several generations and subsequent outcrossing. To this day, they are still sporadically in use [15, 93]. However, we do not see them as adequate alternatives for long-term breeding because inbreeding in the individual lines will increase drastically and after outcrossing in a closed population, the queens of the next generation will be heterozygous but closely related, resulting in accelerated inbreeding in the next generations. Furthermore, one should remark that Page and Laidlaw based their analysis exclusively on considerations about the sex locus *csd*, which they assumed to have between 6 and 19 alleles in a population. In the meantime, it has been shown that this number is in reality at least one order of magnitude higher [94]. We therefore see across-family selection with appropriate selection rates, as presented in this study, as the more promising breeding alternative.

In the future, further improvements could be reached with more sophisticated selection schemes. Optimum contribution selection was designed to maximize genetic gain at a predefined generational inbreeding rate [95]. However, to this day, this concept has not been adjusted to the genetic peculiarities of the honeybee.

Furthermore, we see both potentials and risks in the emergence of genomic selection methods in the honeybee [96–98]. In practice, the introduction of genomic selection has led to increased inbreeding rates and loss of genetic variance in cattle populations [99, 100]. However, it has been proven that genomic breeding can outperform traditional methods in respect to maintenance of genetic variance and effective population size when applied correctly [101–103].

5.5 Conclusion

Sustainable across-family selection of honeybees can be achieved for breeding populations with at least 200 colonies per year. In populations with 200 colonies, sister group sizes should not exceed three to four queens in order to avoid high inbreeding rates. For larger populations (500 to 1000 queens per year), this number can be increased to four to six queens, if a sufficient number of sires can be provided. The number of sires should generally be high, especially in small populations. Breeding schemes that are sustainable in the long term (100 years) may come at the cost of slightly decreased genetic gain in the short term (15 years).

The results we obtained here *in silico* have to be compared to *in situ* experiences that can be collected over the years and regulations should be adjusted accordingly.

5.6 References

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Chapter 6

Conclusion and outlook

This work demonstrates how the use of honeybee-specific Monte Carlo simulations can increase the knowledge about honeybee breeding processes, leading to real life applications. By using the program `BeeSim`, the genetic development was for the first time simulated at the level of individual queens, drones and worker groups (earlier studies had used simplified models of inheritance [1, 2]). Chapters 3, 4, and 5 pursued and found answers to the concrete questions

- What are suitable genetic models to simulate long-term honeybee breeding?
- To what extent is breeding with controlled mating superior to breeding without?
- At which rate should queens be selected to sustainably yield high genetic gain?

Since these were the first simulations with this degree of detail, many observations were made on the side, thus enriching the main findings. In the introduction, four key issues of animal breeding were addressed, viz. *detection*, *decision*, *control* and *sustainability*. I feel confident that this work provides valuable contributions to all four of them. In particular:

- *Detection.* All of the studies showed high rates of genetic gain—provided that mating was controlled. This proves that genetically superior honeybees are indeed detected when bee-specific genetic evaluation is applied. Thus, the simulations serve as a validation of the new concepts developed by Brascamp and Bijma [2] and Bernstein et al. [3]. Furthermore, one could observe how different genetic parameters, and in particular the correlation between maternal and direct effects, influence the accuracies of estimated breeding values (Sections 3.4.2, 3.8, and 4.4.3). The observation that estimated breeding values may be subject to an increasing bias if

changing genetic parameters are not addressed correctly (Section 3.3.4, Figure 3.4) was an important insight to optimize following simulation studies (Section 5.2.3). Moreover, it signifies the importance to re-estimate genetic parameters regularly in practical breeding programs instead of relying on once established genetic parameters. This shows that recommendations for concrete action (repeated parameter estimations) can be derived from the simulation studies to improve the *detection* aspect of honeybee breeding.

- *Decision.* The most common way to treat trade-off decisions between several negatively correlated traits or effects is to select for so-called *indexes*, i. e. weighted sums of the individual components. In particular for indirect effects, such as the omnipresent maternal queen effects in honeybee breeding, it has been shown before that this is the ideal procedure [4]. But while the `BeeSim` simulations did not yield new theory in this regard, they showed consequences which the combined selection for direct and maternal effects have for breeding populations of honeybees. In particular, they showed that for genetic parameters as they are commonly found for economically important traits, the selection focus lies much more on the direct than on the maternal effects (Sections 3.3.1 and 3.8) and that in case of strongly negatively correlated effects, theoretically optimal breeding decisions may even lead to negative genetic developments in the maternal effects (Section 4.3.3, Figure 4.2). As discussed in Section 4.4.3, the consequences of these extreme selection foci in practice are little understood. Since strongly negative correlations between effects have repeatedly been estimated for the honeybee [5, 6], the simulation studies indicate that special attention should be payed to the individual developments of direct and maternal effects and the consequences that breeding *decisions* have on either of them.

- *Control.* The entire Chapter 4 is dedicated to the role of mating *control* in honeybee breeding. The great differences in genetic gain with and without controlled mating make clear that this is a paramount aspect of successful breeding activities and thus gives a clear directive to breeding organizations to make the infrastructure of controlled mating available to their breeders.

- *Sustainability.* While Chapter 3 raises first issues regarding the *sustainability* of breeding values (Section 3.4.2), the topic plays a major role throughout Chapter 5. In this chapter concrete recommendations for the sustainable yet successful design of

breeding schemes were presented, in particular for small and endangered honeybee populations. Specifically, it was shown that testing procedures featuring up to eight sister queens, as they are currently suggested for the large Central European population of *A. m. carnica*, should not be transferred to smaller populations and that sister group sizes should rather be reduced. This is a valuable contribution to the recent and future establishment of breeding programs for endangered honeybee subspecies.

There is great potential for further applications of stochastic simulation studies of honeybee breeding as they are provided by `BeeSim`. The finite locus model can easily be combined with genomic methods of genetic evaluation [7]. The exploration of genomic BLUP methodology for the honeybee by simulation studies can help to optimize procedures for this species. In cattle and swine, the introduction of genomic selection has lead to immense improvements in the *detection* of genetically superior individuals. Now that the necessary SNP panel is available for the honeybee, too, vast improvements are to be expected [8]. Simulation studies help to indicate what can be expected of the new tools and how they can be applied to achieve an optimal outcome.

The interplay between direct and maternal effects in the honeybee are an interesting field that deserves greater attention. Building on what has been observed in the studies presented here, it appears worth exploring how selection practices affect the genetic correlations between effects and how strategies may be developed to prevent negative selection for maternal effects. Furthermore, the inclusion of non-additive effects and multiple traits into the simulations may lead to new insights regarding trade-off *decisions*.

We have already shown that mating *control* is important. In the furture, it should be further examined, how controlled mating can be guaranteed in the best way. Strategies including artificial insemination and isolated mating stations should be tested against one another to find ideal solutions. As we indicated in Section 5.4.2, the rising popularity of artificial insemination provides a wide range of different strategies, the pros and contras of which are thus far only poorly understood.

Also the aspect of *sustainability* can be developed further. The concept of optimal contribution selection [9] should be applied to the honeybee. Once the theoretical framework herefore is set, simulation studies can help with the species-specific calibration.

The numerous new experiences which will be made with the emerging European breeding programs will likely give rise to multiple further questions, the nature of which is not yet

foreseeable. However, the flexible design of the `BeeSim` software gives us confidence that (partial) answers can be found by the means of stochastic simulation studies.
Finally, some applications of `BeeSim` may even be found outside the area of honeybee breeding. With only slight modifications, systems of natural rather than artificial selection may be investigated, giving rise to evolutionary studies for honeybees.

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